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TOXICOLOGICAL AND RECALCITRANT PROPERTIES OF A PROPOSED PROPELL--ETC(U)
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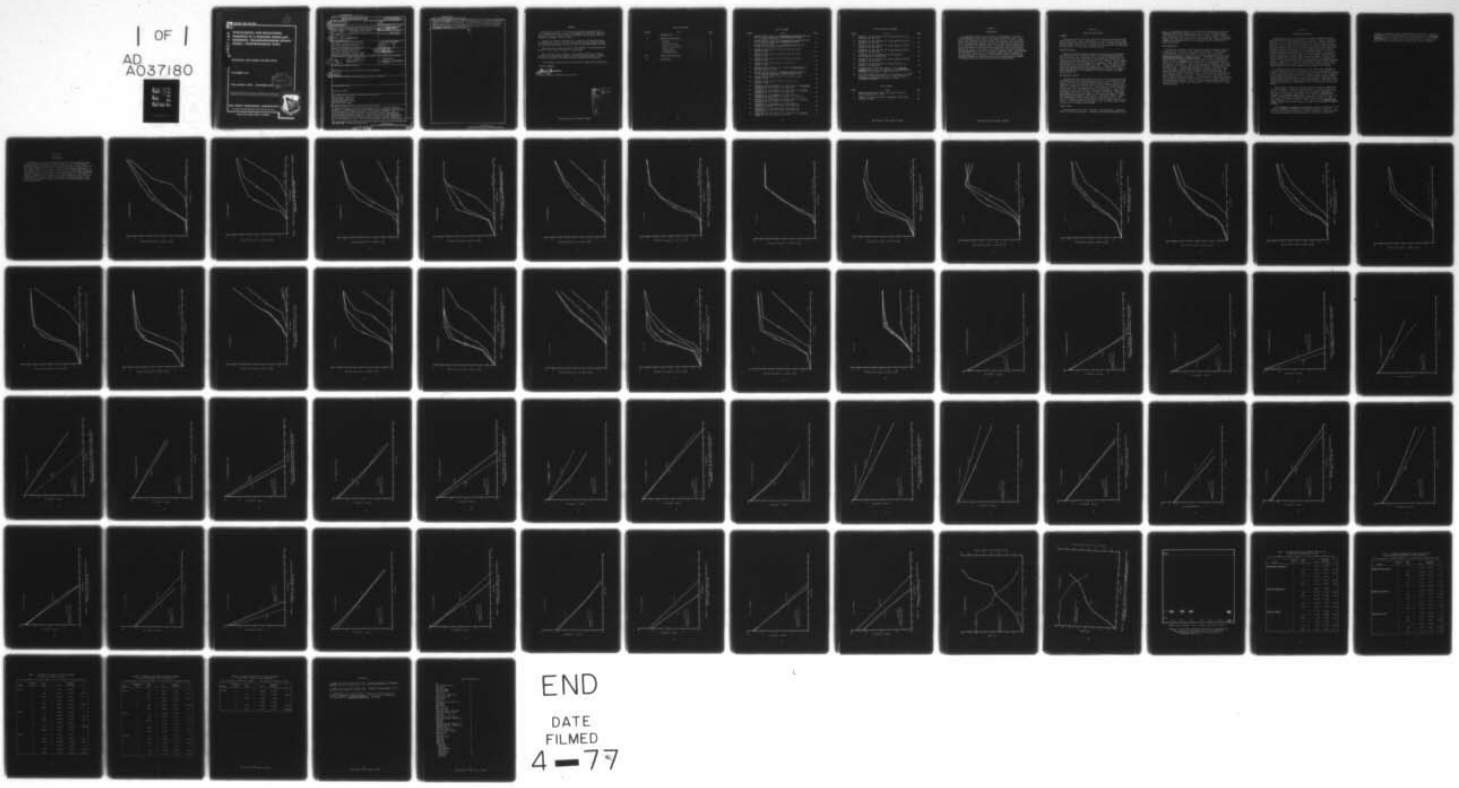

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TOXICOLOGICAL AND RECALCITRANT PROPERTIES OF A PROPOSED PROPELLANT INGREDIENT, TRIAMINOGUANIDINE NITRATE (TAGN) I. MICROBIOLOGICAL STUDY

ENVIRONICS AND HUMAN FACTORS OFFICE

NOVEMBER 1976

FINAL REPORT: APRIL - NOVEMBER 1976

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AIR FORCE ARMAMENT LABORATORY
AIR FORCE SYSTEMS COMMAND • UNITED STATES AIR FORCE
EGLIN AIR FORCE BASE, FLORIDA

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Triaminoguanidine Nitrate
Microbial Populations

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The toxicological and recalcitrant properties of a proposed propellant ingredient, triaminoguanidine nitrate (TAGN), were investigated. Pure cultures of microorganisms isolated from Eglin Air Force Base, Florida, as well as cultures obtained from US Army Natick Laboratories, Natick, Massachusetts were exposed to TAGN and evaluated. During the course of this investigation, it was determined that microbial populations were not adversely affected by short-term exposure to TAGN. The following parameters were not significantly

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(Item 20 concluded) altered by TAGN concentrations up to 50 ppm: growth rate, respiratory activity, and viability. At concentrations greater than 100 ppm, TAGN was bacteriostatic but not bacteriocidal. Of the two bacteria tested, Pseudomonas aeruginosa and Escherichia coli, both were capable of removing (degrading) TAGN from aqueous solution.

PREFACE

This technical report is the result of research conducted by the Air Force Armament Laboratory, Armament Development and Test Center, Eglin Air Force Base, Florida, from April 1976 to November 1976 under Air Force Exploratory Development Project 50660101.

Reference to specific manufacturers or suppliers of scientific equipment used in this study is for the sole purpose of identification and does not constitute endorsement of these products by the United States Air Force.

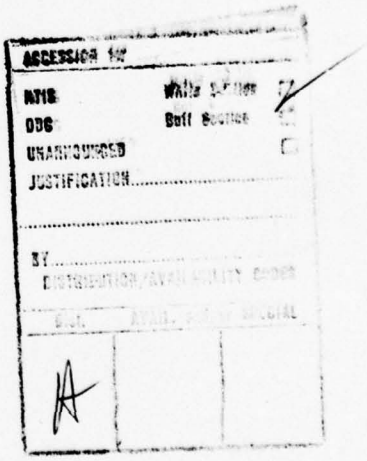
The assistance of Cadet Ron Alford, USAF Academy, in the bacteriocidal portion of this study is gratefully acknowledged.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER:

JOE A. FARMER
Chief, Environics and Human Factors Office



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SECTION I

INTRODUCTION

An experimental propellant consisting of approximately 45 percent triaminoguanidine nitrate (TAGN), 19 percent nitrocellulose (NC), 30 percent cyclotetramethylene tetranitramine (HMX), 5 percent isodecyl pelargonate, and 1 percent resorcinol is being considered by the Air Force for use in gun ammunition employing high-density, armor-piercing penetrators. It is a matter of environmental policy to determine toxicity and evaluate methods for disposal of new propellant constituents before inventory acquisitions. Prior to this study, no information was available concerning the biodegradation or toxicity of the major component, TAGN. The objectives of this initial study were to investigate whether TAGN is degradable by microorganisms and to determine if TAGN adversely affects microbial populations indigenous to soil and water habitats where appreciable amounts of TAGN may accumulate during propellant testing and disposal.

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SECTION II

MATERIALS AND METHODS

CULTURES

Bacterial strains used in this study were obtained either from US Army Natick Laboratories, Natick, Massachusetts, or isolated from soil or water samples collected at Eglin Air Force Base, Florida. Original cultures were preserved under liquid nitrogen. Subcultures were maintained on Trypticase soy agar (TSA) at 4°C and were transferred monthly.

INHIBITORY STUDIES

Starter cultures were grown overnight in Trypticase soy broth (TSB) with agitation at 20°C and were diluted 1:10 in 15-mM phosphate buffer (pH 6.6) prior to use. Experiments were initiated by inoculating 0.1-mℓ cells into 5.0-mℓ filter-sterilized (0.45-μm Millipore filters) TSB containing TAGN at concentrations of 500, 100, 50, or 10 ppm. Control samples contained no TAGN. All experiments were performed in triplicate at 20°C with agitation on a gyratory shaker (120 rpm). Growth was monitored periodically by recording the optical density (O.D.) of each sample with a Bausch and Lomb Spectronic 20 Spectrophotometer at 520 nm.[1] All samples were corrected for O.D. discrepancies due to tube variations and TAGN-induced absorption.

BACTERIOCIDAL EFFECTS

Cultures were grown overnight in 50-mℓ TSB at 25°C with agitation and were harvested at late log or stationary phase by centrifugation at 6,000 rpm for 15 minutes in an IEC/B20 refrigerated centrifuge. Following resuspension in sterile phosphate buffer, the cells were diluted to give a final O.D. reading of 0.2 at 520 nm. Diluted samples (5.0 mℓ) were added in duplicate to 5.0-mℓ phosphate buffer containing TAGN at final concentrations of 0, 500, and 2,000 ppm. Samples were shaken in sterile 15-mℓ centrifuge tubes at 25°C for 1-hour or 5-hour time periods. Afterwards, the exposed cells were centrifuged at 6,000 rpm for 15 minutes to remove TAGN and were resuspended in equal amounts of phosphate buffer. Samples were subsequently diluted with buffer to a final titer of 3.0×10^2 - 3.0×10^3 colony-forming units/mℓ (CFU/mℓ) and were spread plated in triplicate. Following incubation overnight at 34°C, the plates were counted with the aid of a Quebec Colony Counter.

OXYGEN UPTAKE

Cultures were grown at 25°C, harvested, and resuspended in phosphate buffer as previously described. Endogenous preparations contained 1.5-mℓ

cells, 1.5-ml phosphate buffer, and a final TAGN concentration of 500 ppm. Exogenous preparations contained 1.5-mℓ cells, 0.5-mℓ phosphate buffer, 1.0-mℓ TSB, and a final TAGN concentration of 500 ppm. Exogenous samples were pre-incubated 30 minutes at 30°C prior to data collection. All control samples were identically prepared but contained no TAGN. Oxygen uptake measurements were performed with a YSI 5331 Oxygen Probe at 30°C with air-saturated solutions.

TAGN DEGRADATION

Twelve liter fermentors (Virtis Research Equipment, Gardiner, New York) containing 10 ℓ of a mineral salts medium consisting of 1.79 g/ℓ KH_2PO_4, 1.65 g/ℓ $Na_2HPO_4 \cdot 7H_2O$, 0.12 g/ℓ $MgSO_4$, 0.03 g/ℓ $CaCl_2$, and 5.0 g/ℓ glucose were inoculated with 25 mℓ of an actively growing culture of either Pseudomonas aeruginosa or Escherichia coli.[2] Immediately following inoculation, filter-sterilized TAGN was added to give a final concentration of 61 ppm for the P. aeruginosa culture and 75 ppm for the E. coli culture. $NaNO_3$ (0.7 g/ℓ) was added to each fermentor at a designated time following inoculation. Periodically, samples were aseptically withdrawn, centrifuged at 6,000 rpm for 15 minutes, and analyzed for TAGN by a modification of the ninhydrin assay.[3] Freshly prepared ninhydrin reagent (3.0 mℓ) was added to 1.0-mℓ sample and heated for 5 minutes in a boiling water bath. The sample was cooled, and the resulting optical density was determined at 570 nm against a blank containing no TAGN. Culture densities were measured prior to centrifugation at 520 nm. In order to rule out the effects of other ninhydrin reacting substances, 50 µℓ supernatant samples were spotted on silica gel GF thin-layer plates (Analtech, Inc., Pittsburgh, Pennsylvania) and were developed against a solvent system of methanol-water-methyl sulfoxide (40:30:30). Plates were sprayed with 0.2 percent ninhydrin in water-saturated butanol.

SECTION III

RESULTS AND DISCUSSION

Growth of the majority of the bacterial isolets examined was not adversely affected by TAGN concentrations up to 50 ppm. However, at 100 ppm and above, 16 of the 22 bacterial cultures tested were markedly inhibited (Figures 1 to 8). Since continued incubation of these cultures for up to seven days did not result in further growth initiations, it must be assumed that the inhibitory effects of TAGN were absolute and not merely the result of greatly extended lag periods. Of the 6 remaining bacterial cultures capable of growth in the presence of 100 ppm TAGN (Figures 9 to 11), all exhibited prolonged lag phases prior to logarithmic growth. Although the overall growth rates of these cultures were considerably retarded, the ultimate cell densities attained, when compared to controls, were not significantly affected by exposure to TAGN. At the present time, inadequate evidence is available to explain the inhibitory actions of TAGN on bacterial cultures. However, it is clear that the inhibitory effects were not due to TAGN-induced pH changes, since the addition of this substance had no pronounced influence on initial hydrogen ion concentration of the media.

To determine whether TAGN was bacteriocidal, cells were suspended in buffered solutions of TAGN at concentrations of 500 or 2,000 ppm for up to 5 hours. These concentrations were high enough to prevent cell proliferation in a suitable medium such as TSB, but as shown in Tables 1 and 2, viability of the cultures was unaffected. In every case the bacteria were capable of renewed growth following TAGN removal by centrifugation. Therefore, while TAGN was bacteriostatic under the specified conditions of this test, it was neither bacteriocidal nor significantly toxic to the microbial cultures examined.

Oxygen uptake, a method of evaluating cellular oxidative capabilities, was investigated at a constant TAGN concentration of 500 ppm (Figures 12 to 25). In several instances oxygen uptake was markedly depressed by 500 ppm TAGN, as in the case of the common soil inhabitants Arthrobacter sp. (Figure 17) and Bacillus cereus (Figure 18). But the majority of the bacteria tested were not significantly influenced by exposure to TAGN. In most cases, the bacteria assimilated oxygen at nearly identical rates to those determined for the controls. A few isolets, such as Staphylococcus aureus (Figure 14) and SR 406 (Figure 21), were even stimulated by exposure to TAGN.

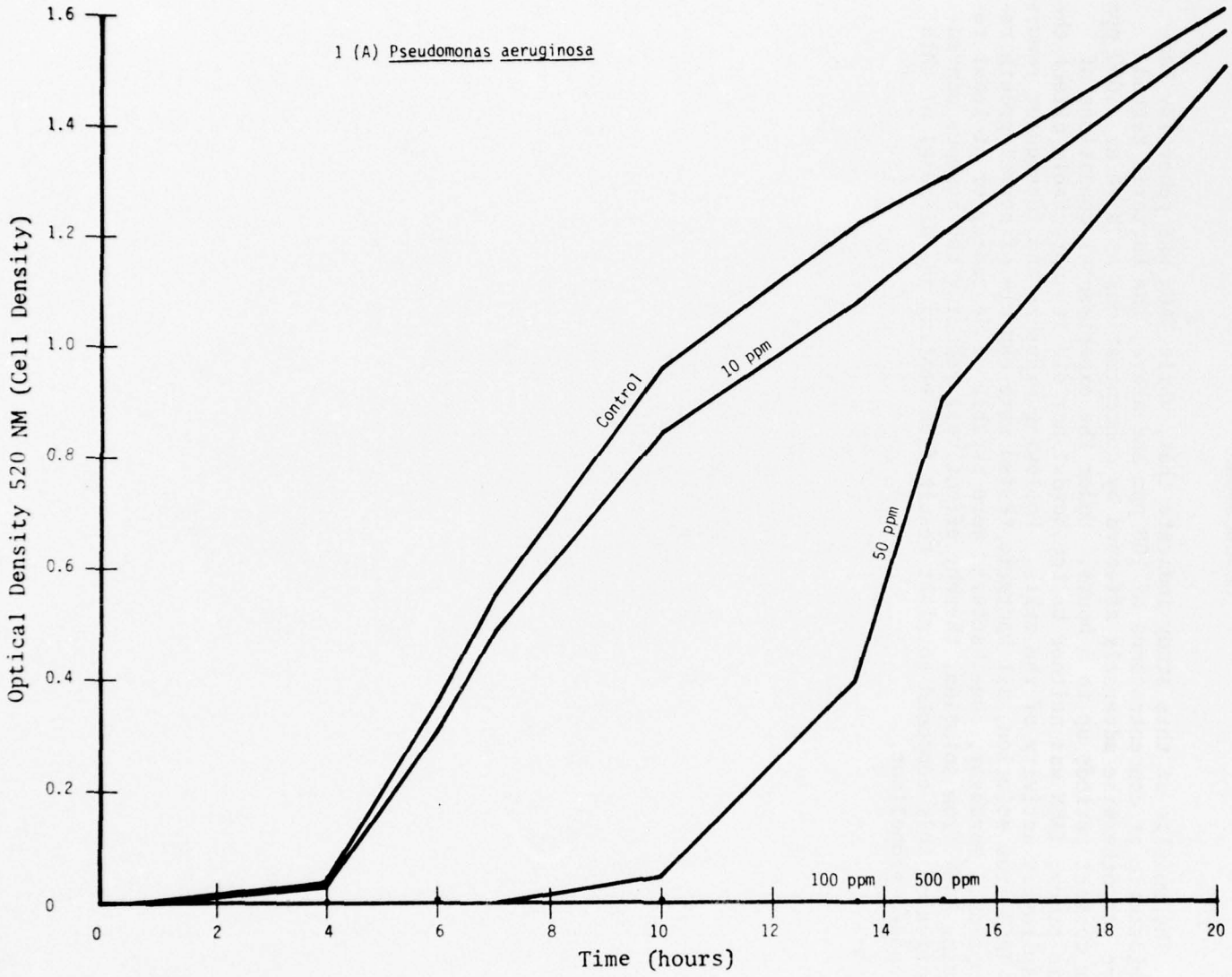
Both Pseudomonas aeruginosa and Escherichia coli were examined for their ability to degrade TAGN under batch fermenter conditions (Figure 26). In each case the bacteria significantly reduced the quantity of TAGN available in solution, persumably through degradation, although no methods were

available to determine active bioaccumulation or adsorption. Thin-layer chromatography of the resulting cell-free supernatants of the Pseudomonas aeruginosa culture (Figure 27) failed to show the presence of any soluble TAGN byproducts, but did provide an additional method to verify the reduction of TAGN in aqueous solutions under these culture conditions.

SECTION IV

CONCLUSIONS

The results of this study indicate that, while TAGN was generally bacteriostatic at concentrations of 100 ppm and above, the bacteria tested were not otherwise adversely affected by concentrations as high as 2,000 ppm for contact periods up to 5 hours. Under the experimental conditions of this study, TAGN was neither bacteriocidal nor did it appreciably affect the respiratory activity of the cells. Following exposure and subsequent removal of TAGN from solution, all bacteria tested were capable of normal growth resumption. Moreover, some bacteria were capable of degrading or at least removing TAGN from solution, thereby effectively reducing the aqueous concentration of this compound as might result from testing and disposal of this proposed propellant.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Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
10 ppm
50 ppm
100 ppm
500 ppm
0
2
4
6
8
10
12
14
16
18
20
Time (hours)

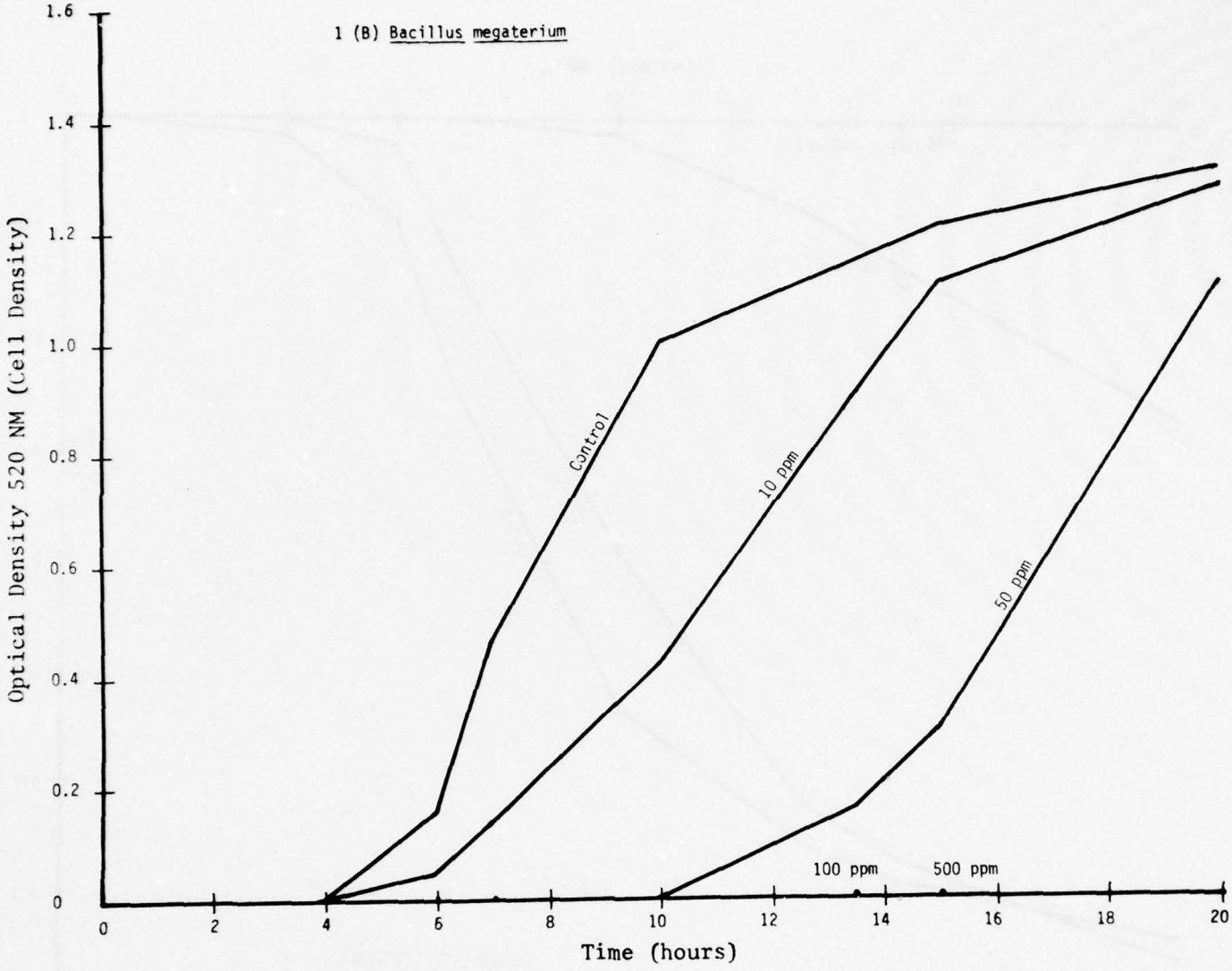
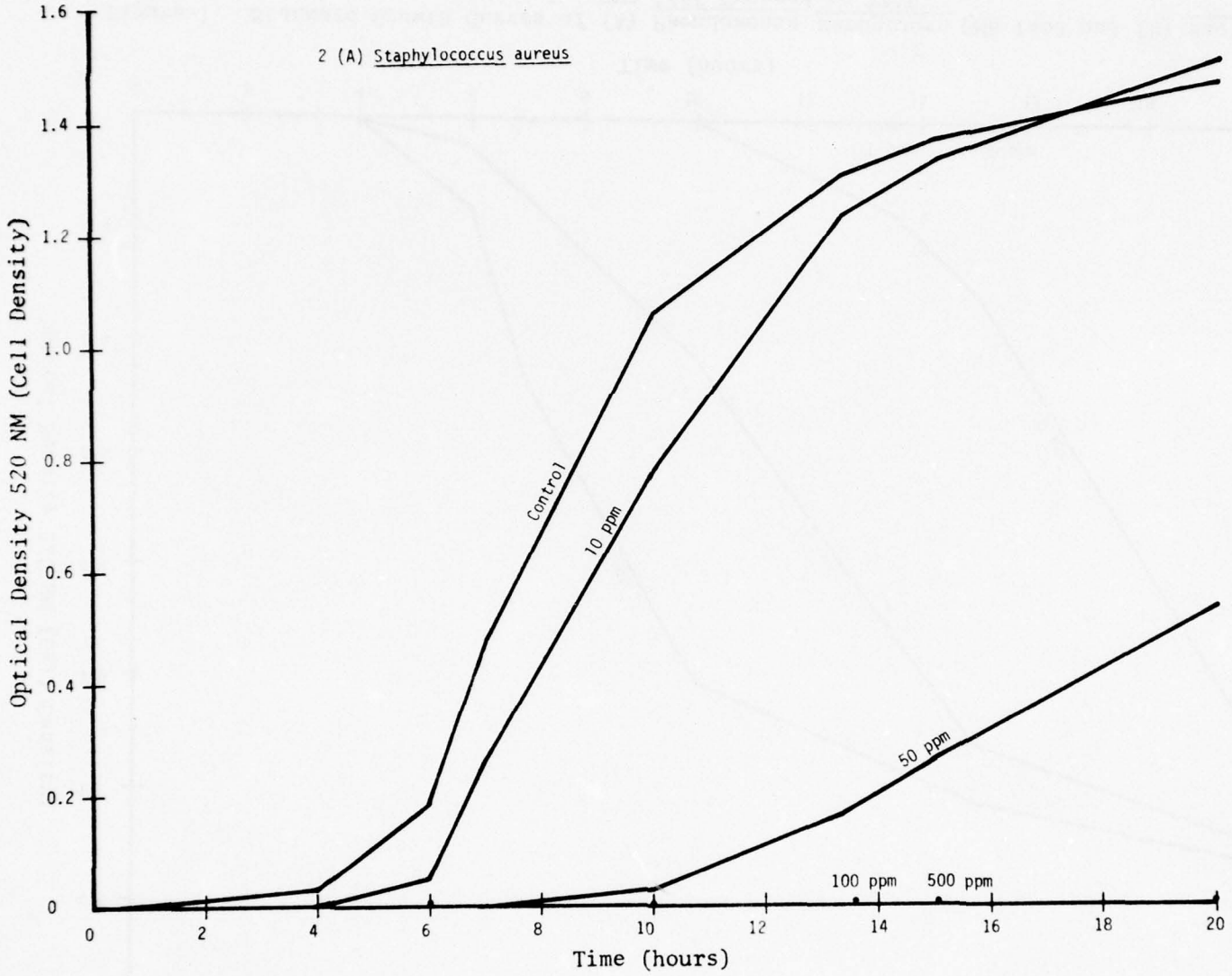


Figure 1. Standard Growth Curves of (A) Pseudomonas aeruginosa QMB 1468 and (B) Bacillus megaterium QMB 1605 Exposed to TAGN


2 (A) Staphylococcus aureus
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
10 ppm
50 ppm
100 ppm
500 ppm
0
2
4
6
8
10
12
14
16
18
20
Time (hours)

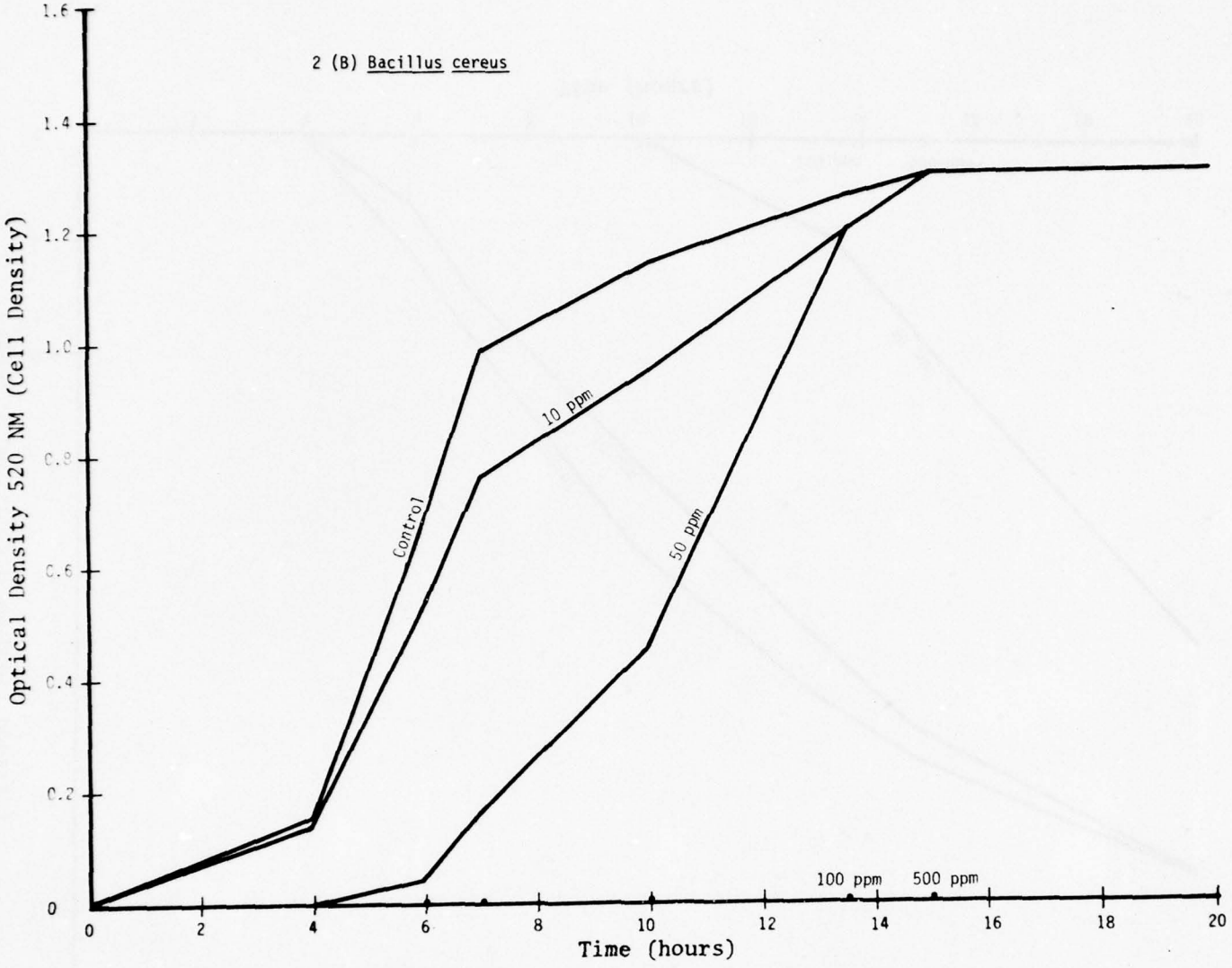
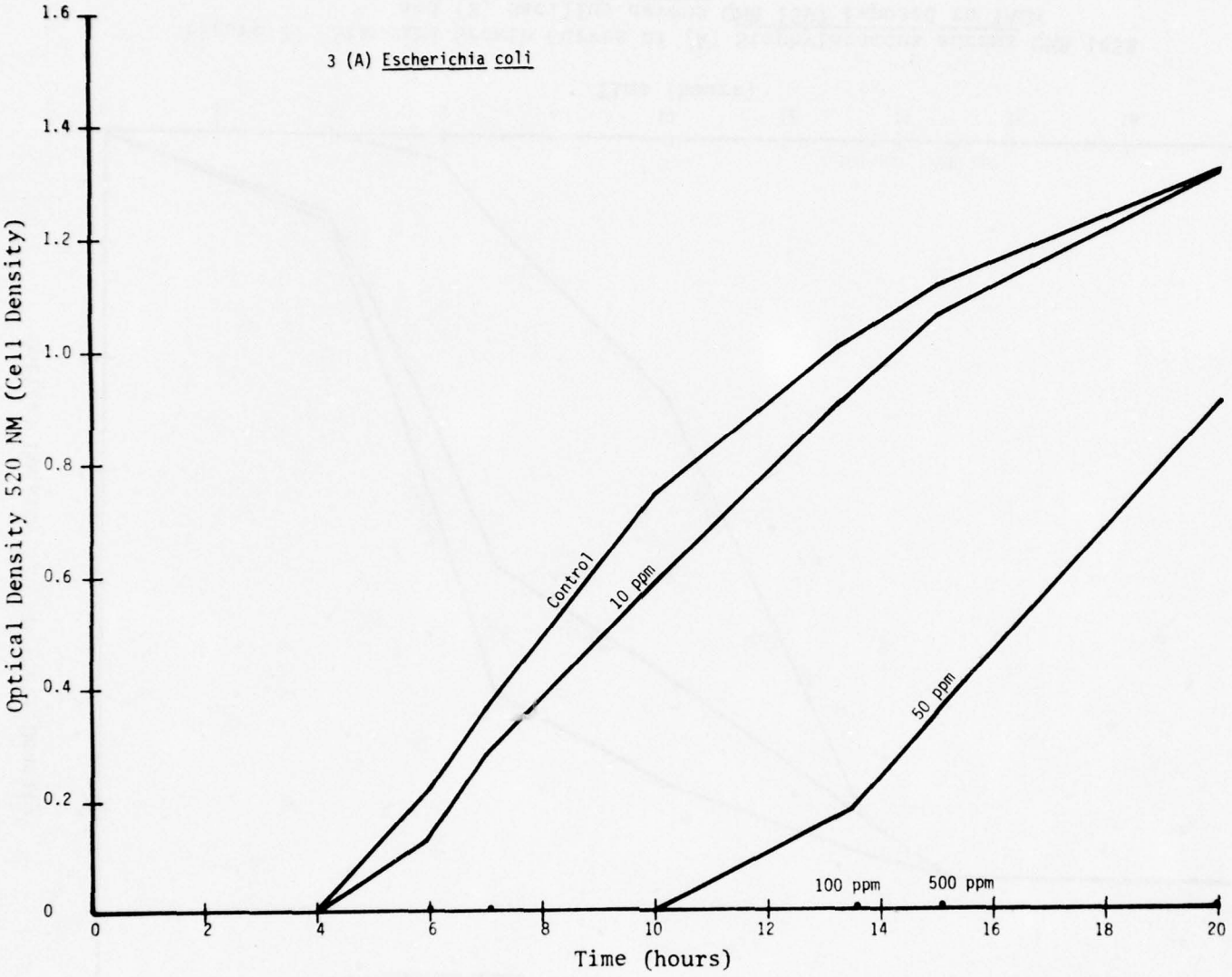


Figure 2. Standard Growth Curves of (A) Staphylococcus aureus QMB 1458 and (B) Bacillus cereus QMB 1597 Exposed to TAGN

3 (A) Escherichia coli



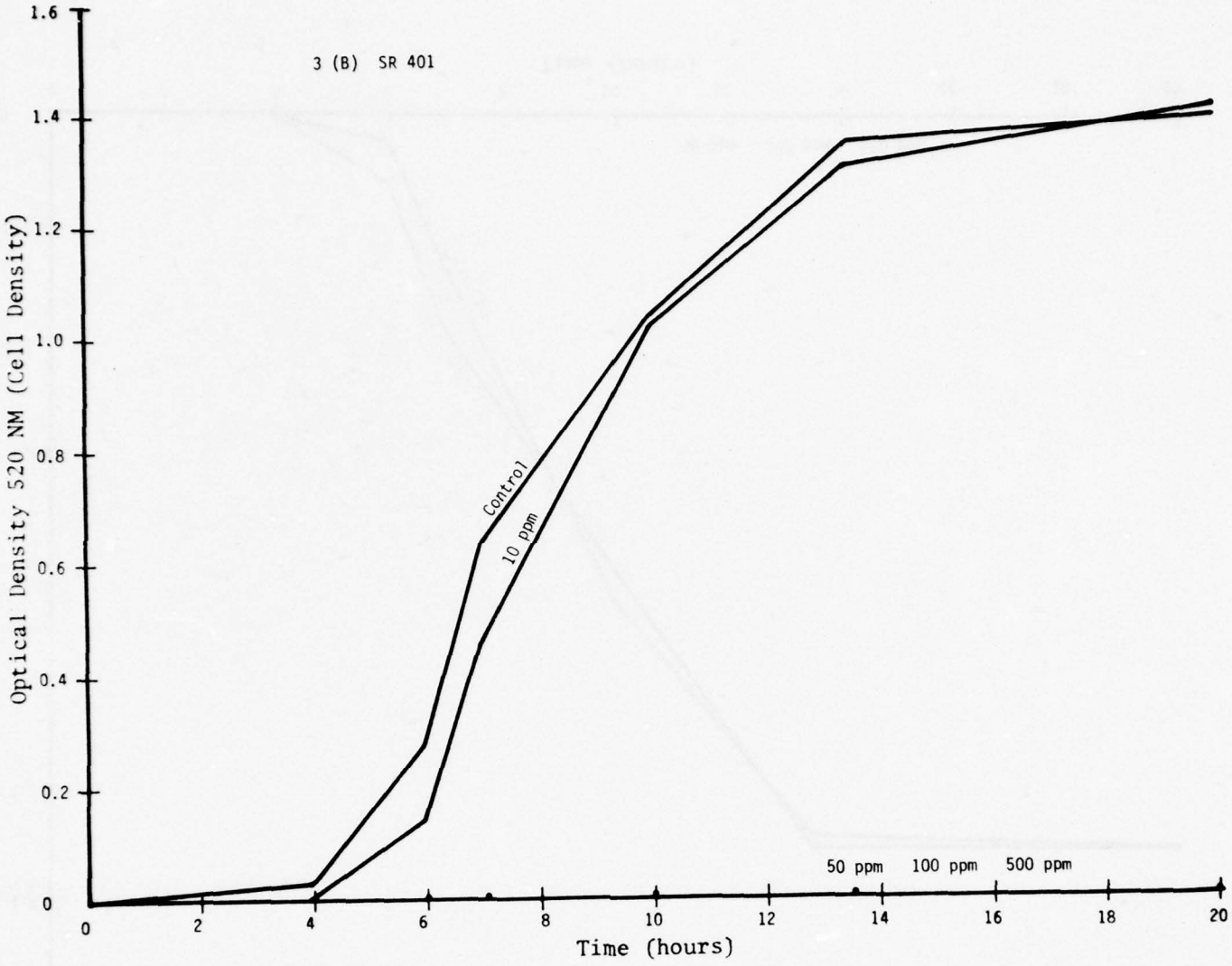
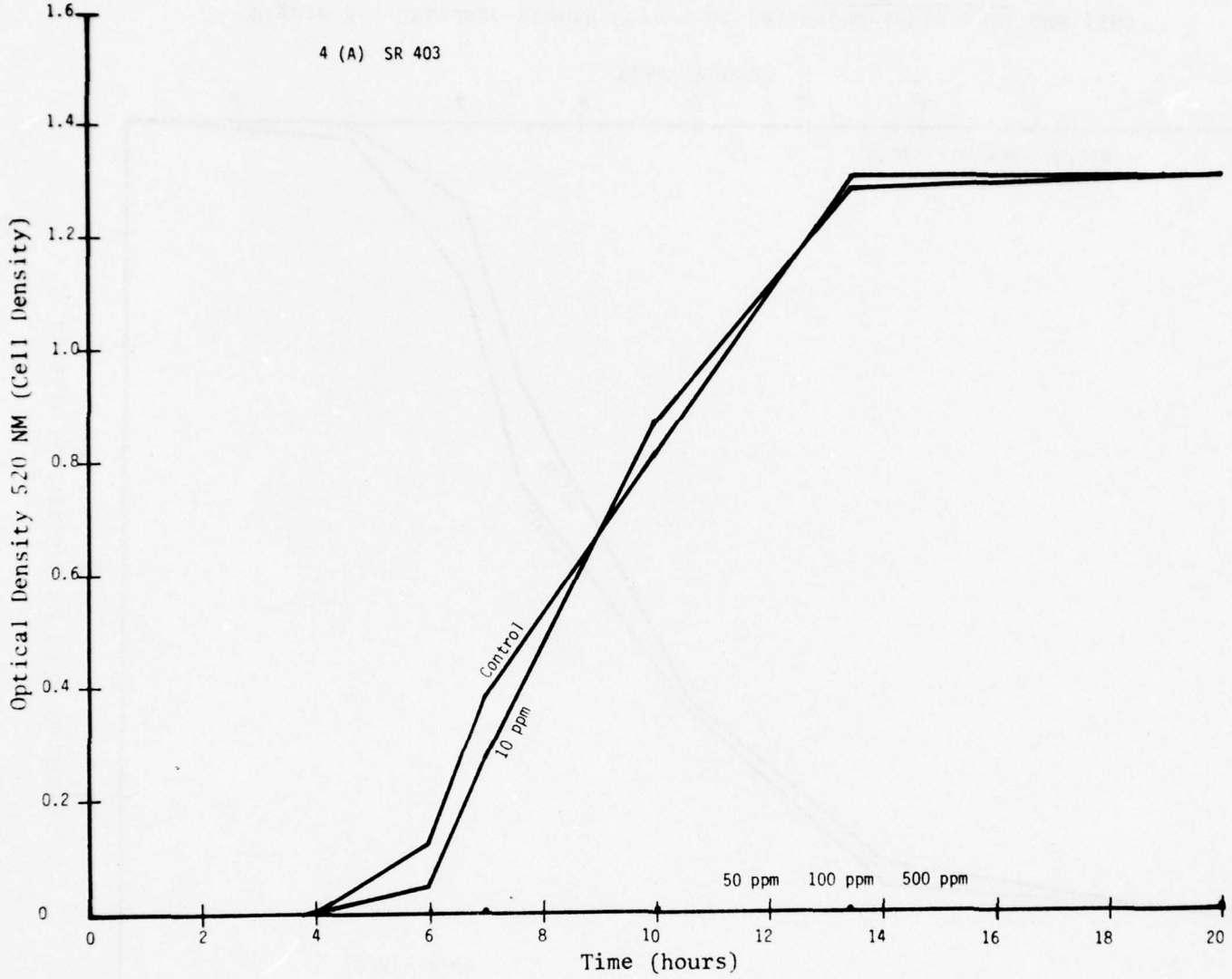


Figure 3. Standard Growth Curves of (A) Escherichia coli QMB 1557 and (B) SR 401 Exposed to TAGN


4 (A) SR 403
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
10 ppm
50 ppm
100 ppm
500 ppm
0
2
4
6
8
10
12
14
16
18
20
Time (hours)

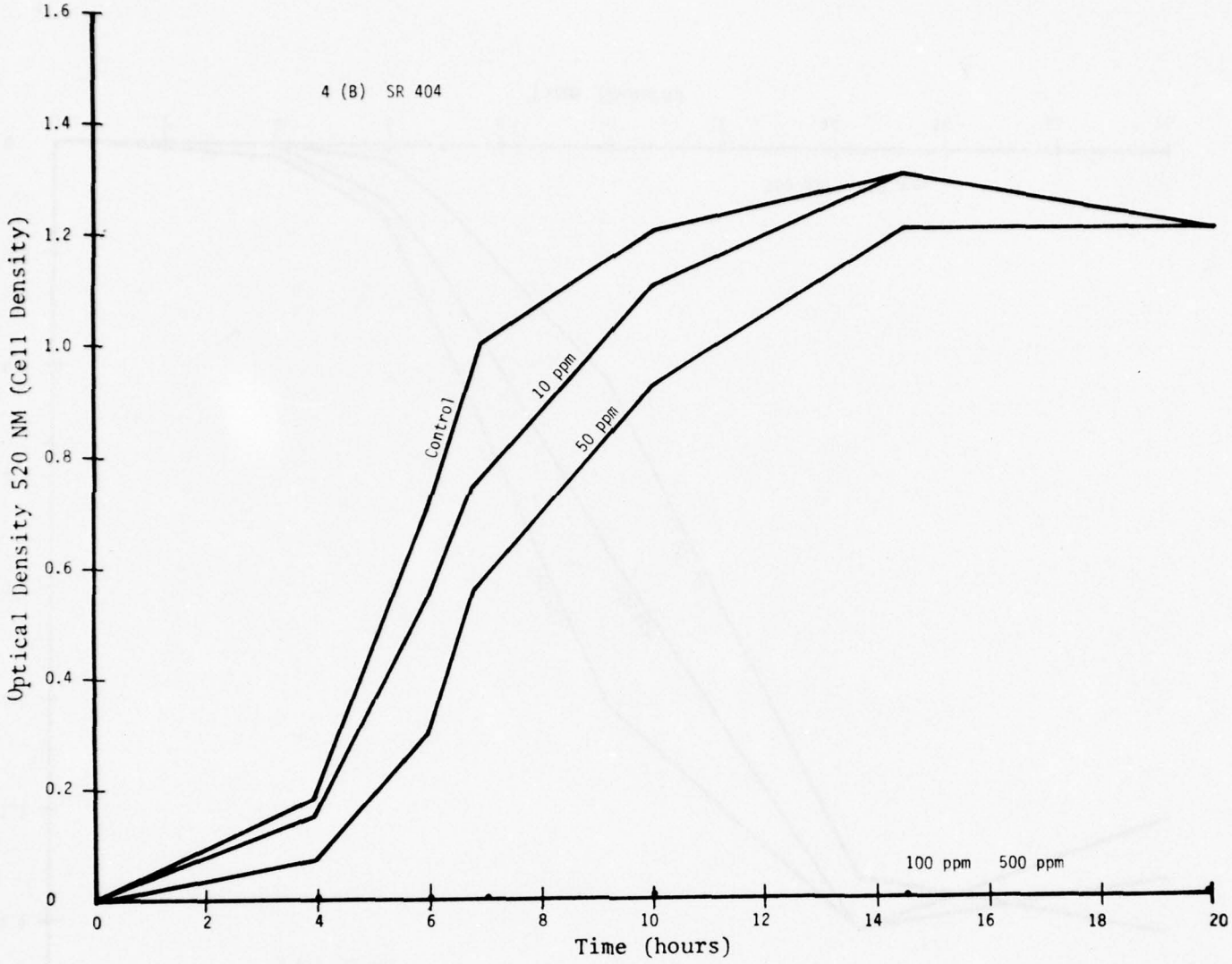
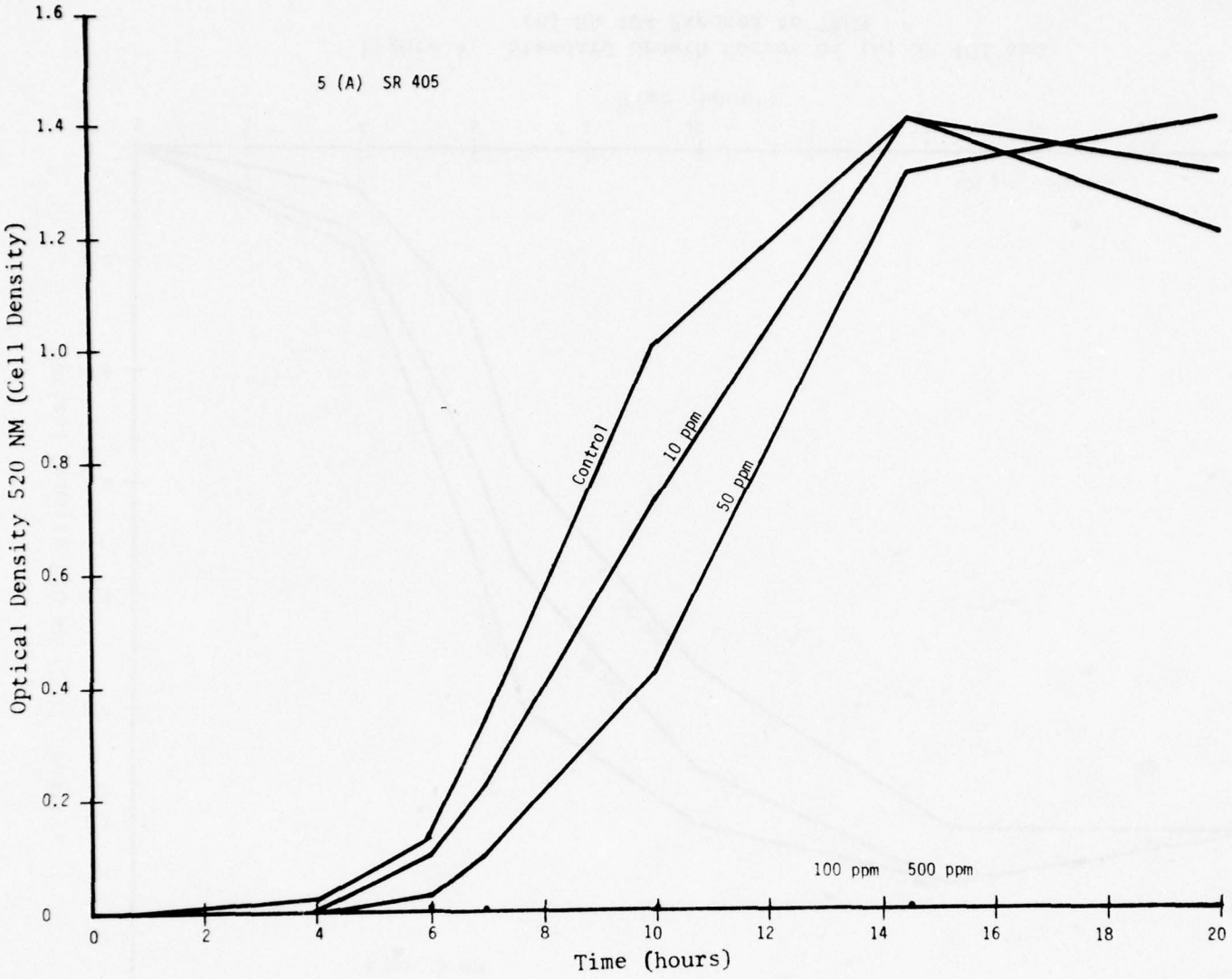


Figure 4. Standard Growth Curves of (A) SR 403 and (B) SR 404 Exposed to TAGN


5 (A) SR 405
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
10 ppm
50 ppm
100 ppm
500 ppm
0
2
4
6
8
10
12
14
16
18
20
Time (hours)

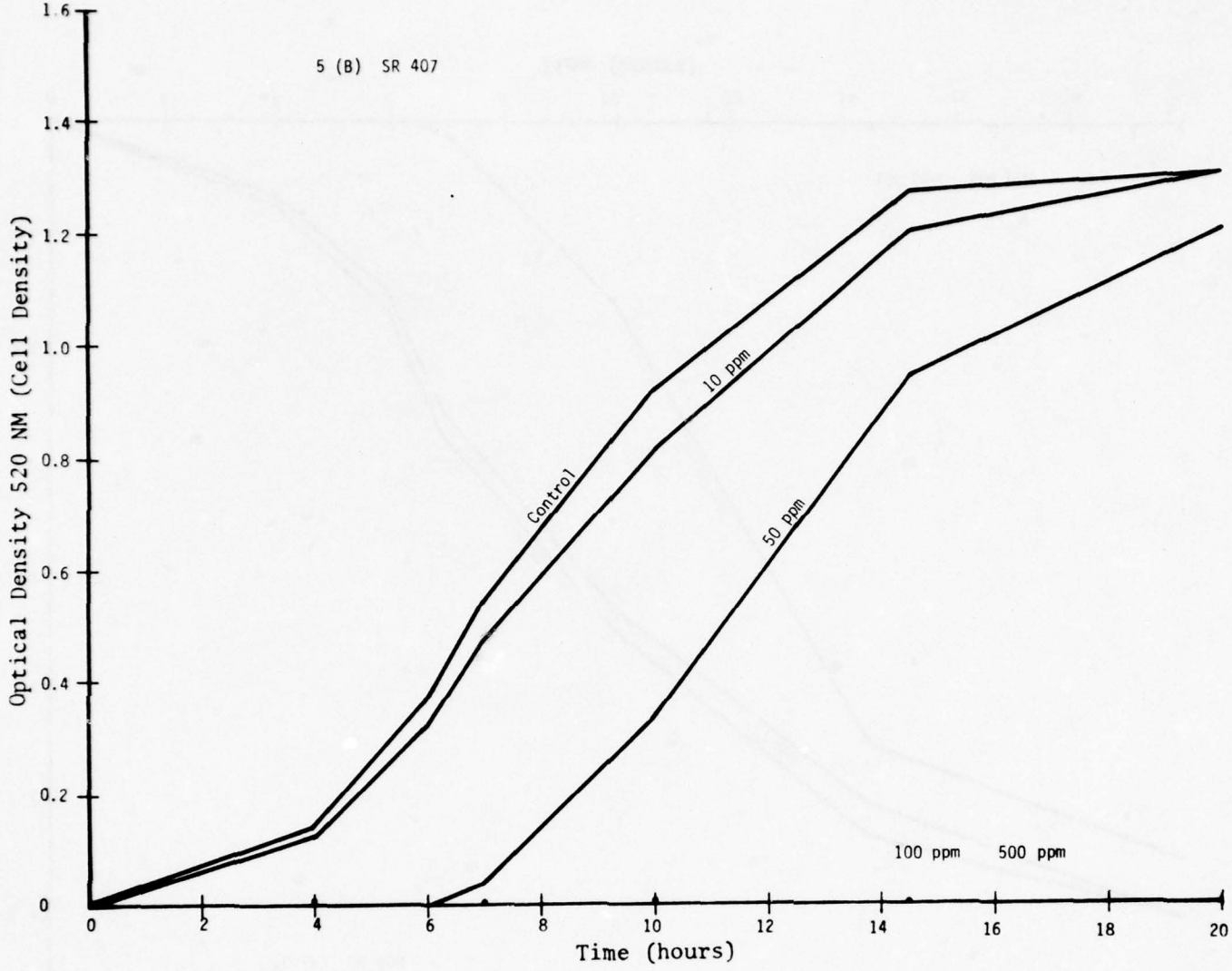
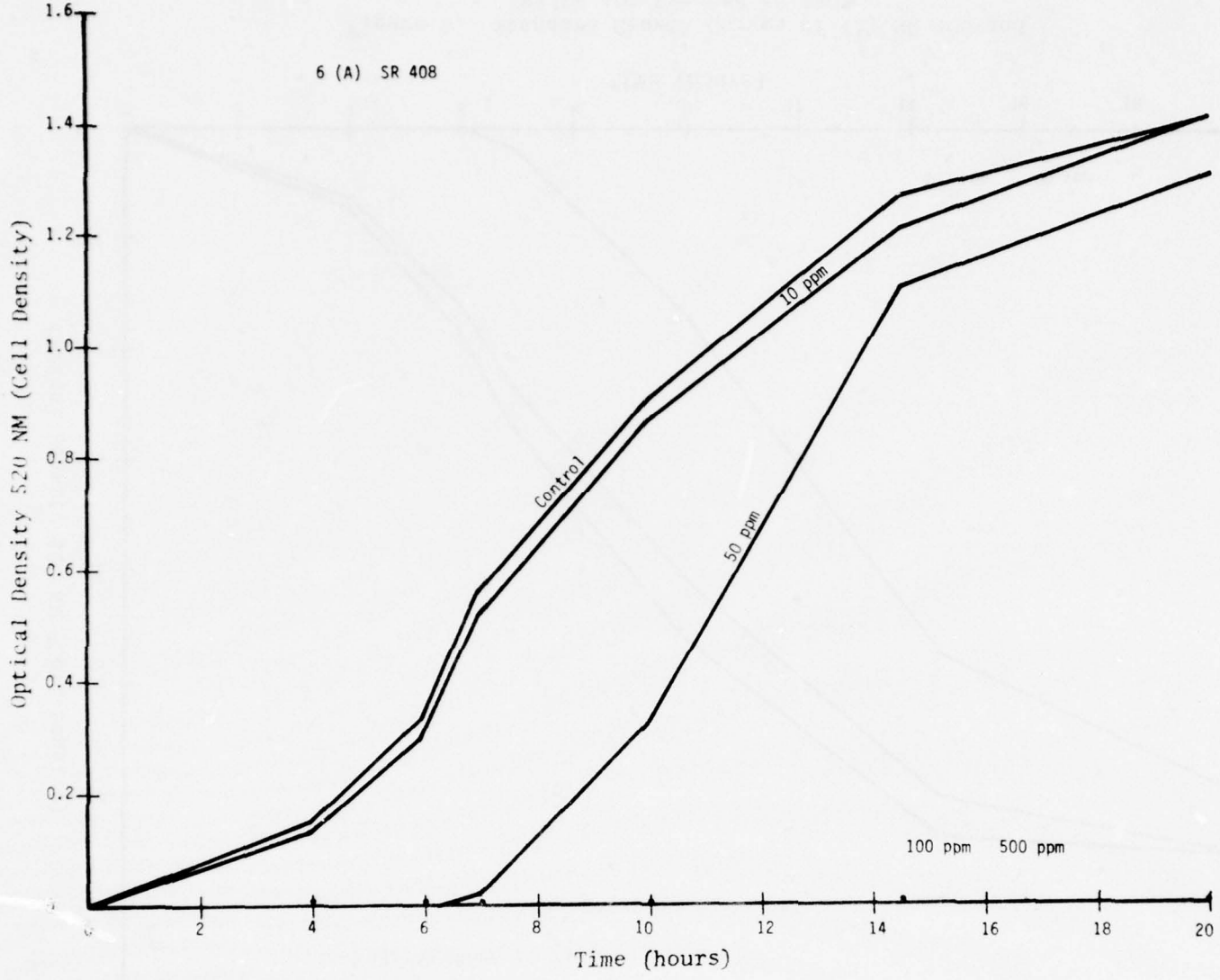


Figure 5. Standard Growth Curves of (A) SR 405 and (B) SR 407 Exposed to TAGN


6 (A) SR 408
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
Control
10 ppm
50 ppm
100 ppm
500 ppm

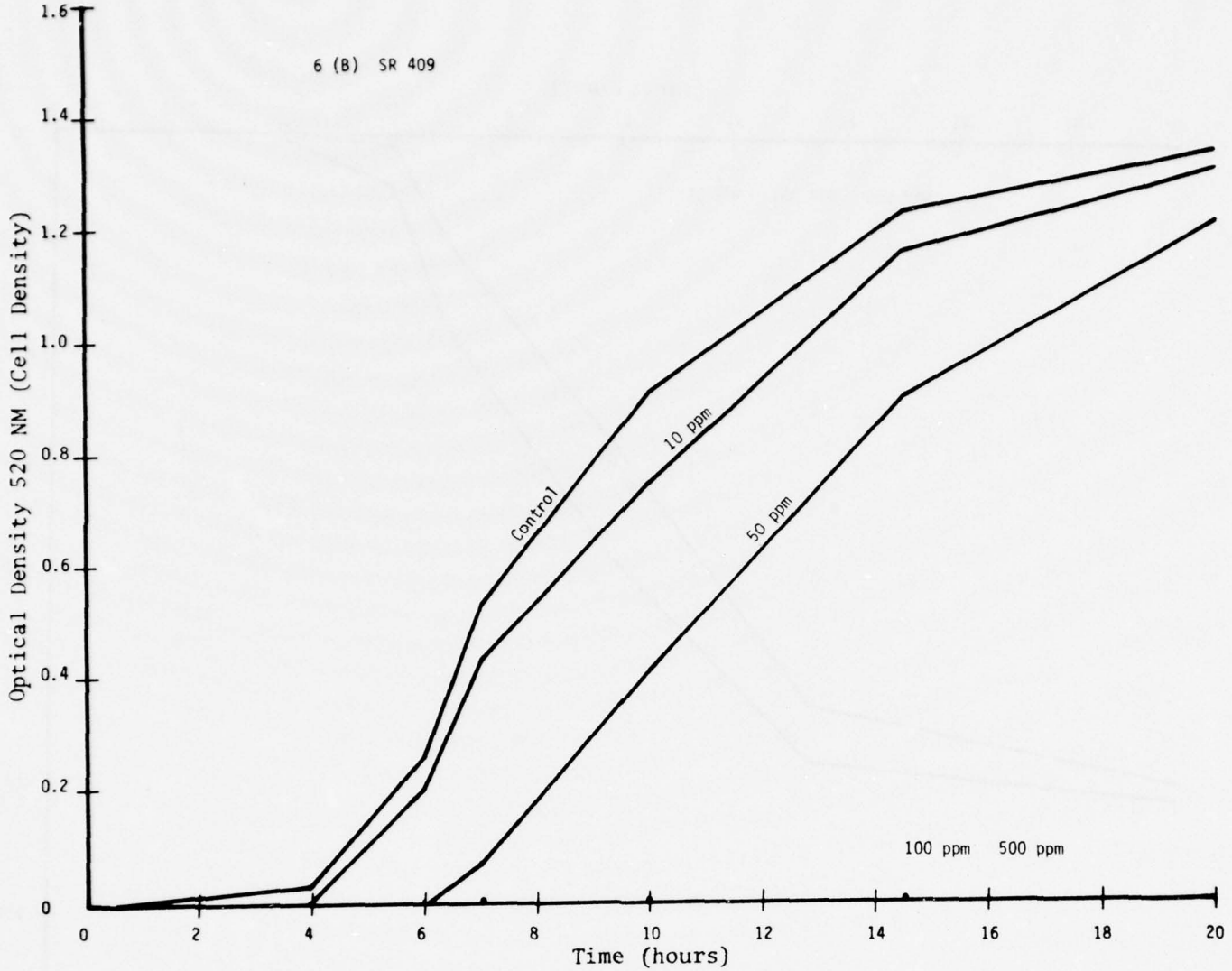
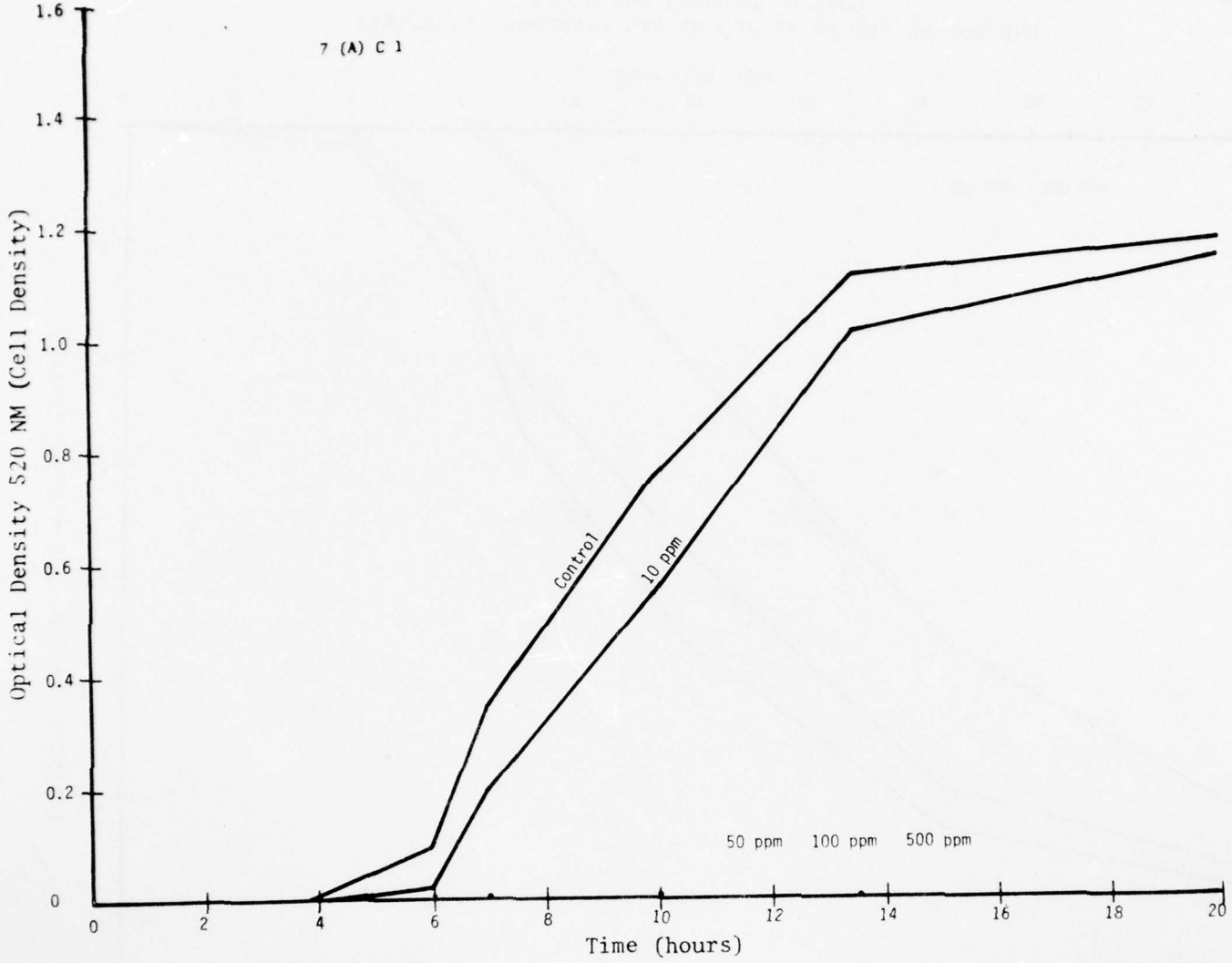


Figure 6. Standard Growth Curves of (A) SR 408 and (B) SR 409 Exposed to TAGN


7 (A) C 1
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
10 ppm
50 ppm
100 ppm
500 ppm
0
2
4
6
8
10
12
14
16
18
20
Time (hours)

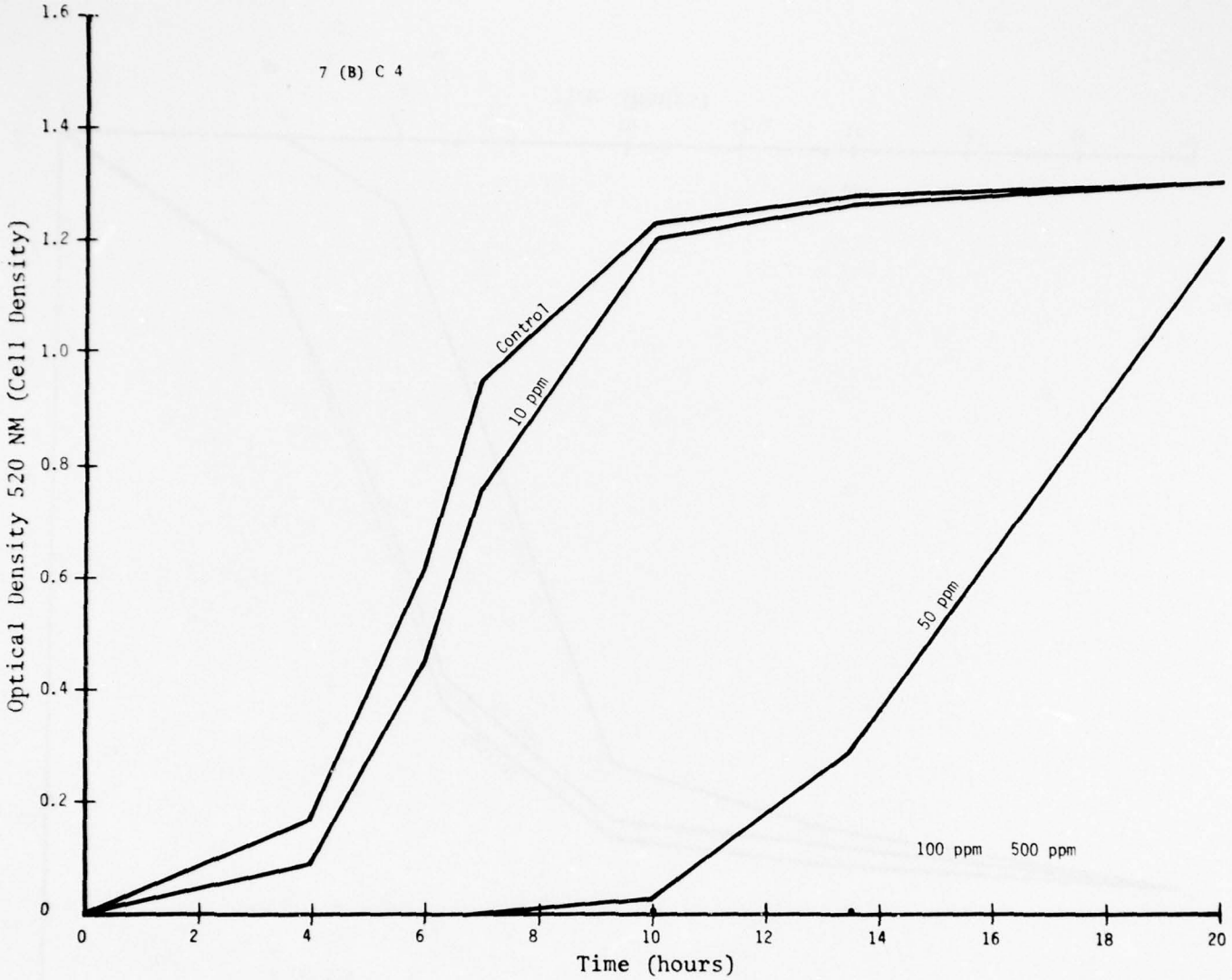
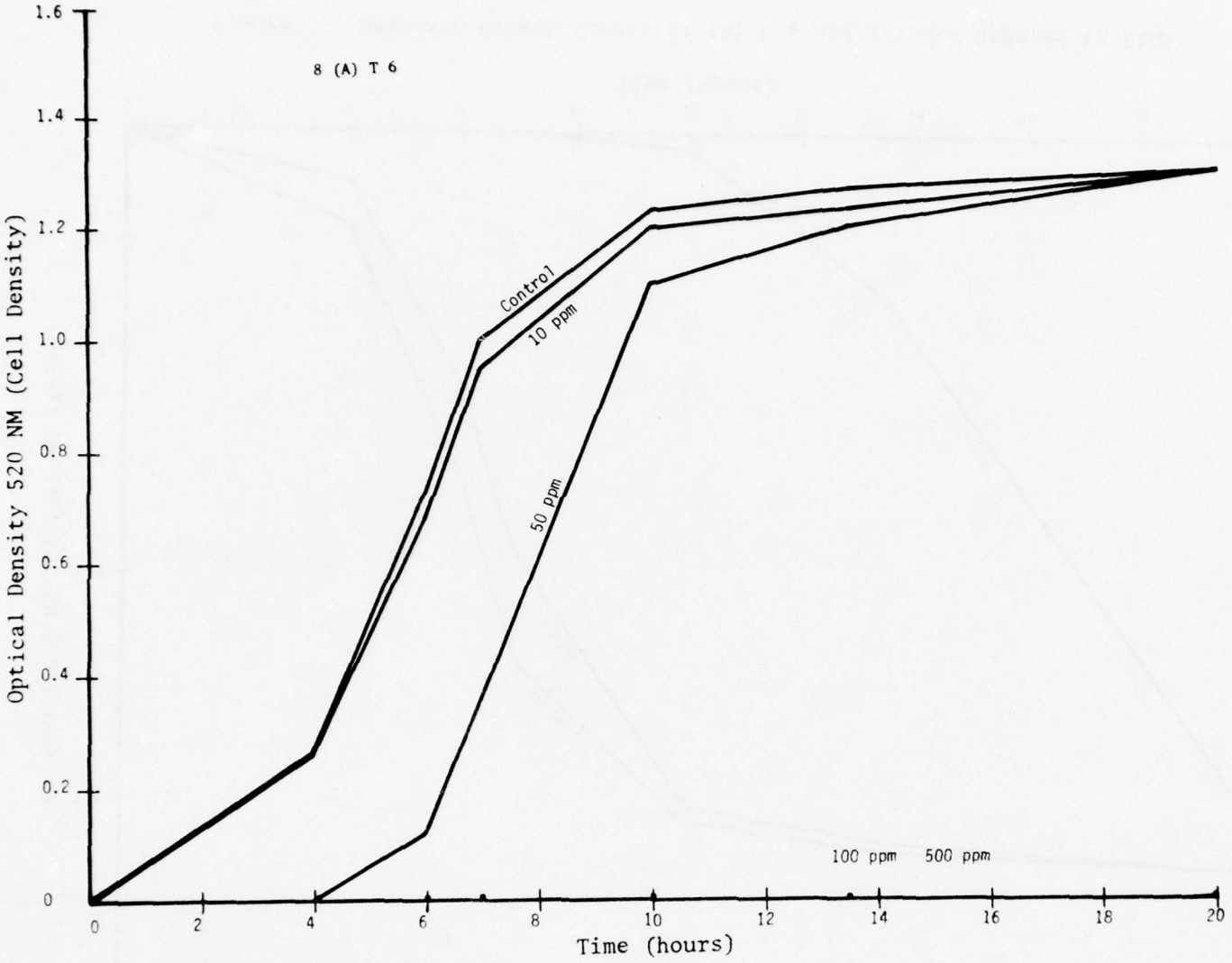


Figure 7. Standard Growth Curves of (A) C 1 and (B) C 4 Exposed to TAGN


8 (A) T 6
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
10 ppm
50 ppm
100 ppm
500 ppm
0
2
4
6
8
10
12
14
16
18
20
Time (hours)

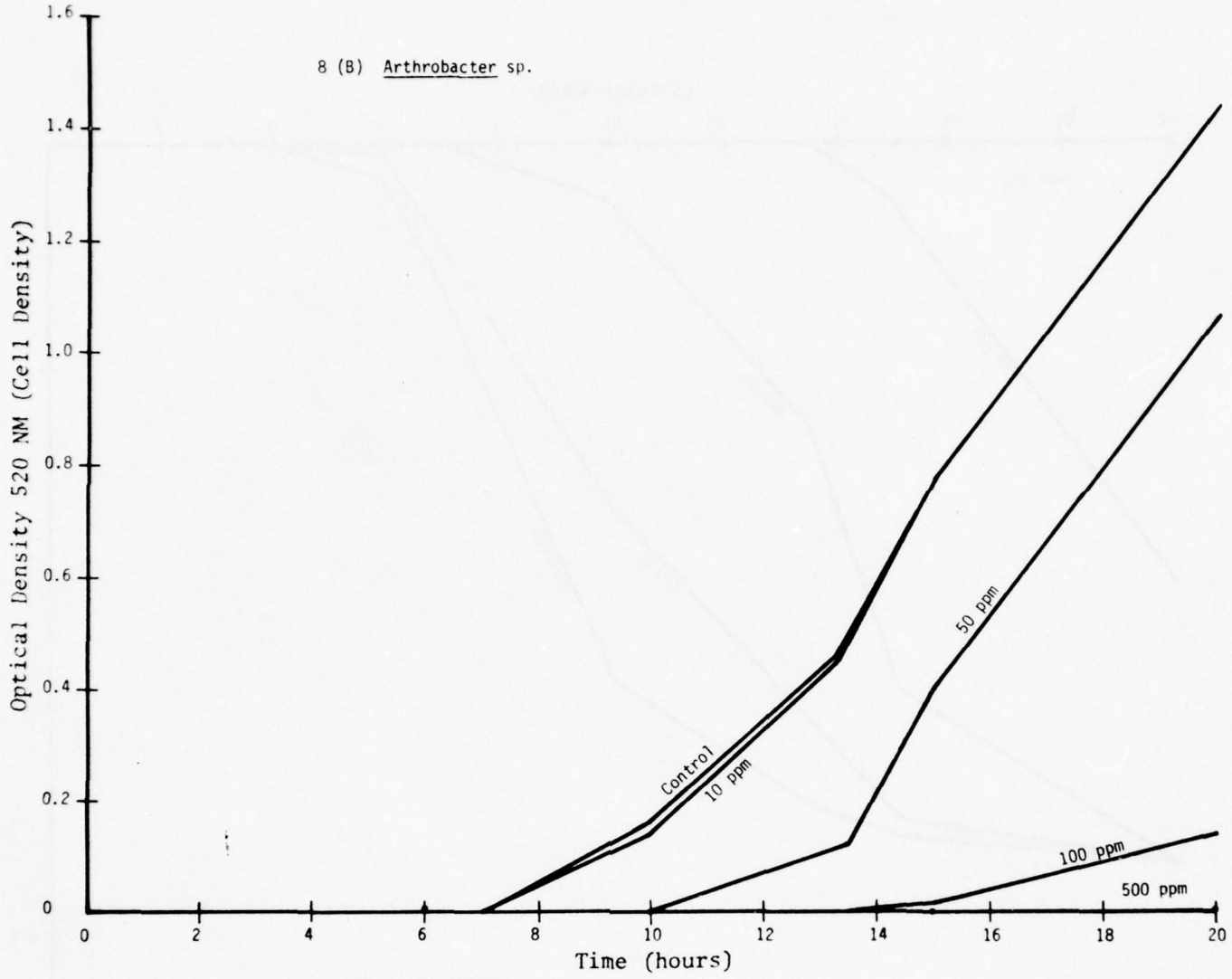
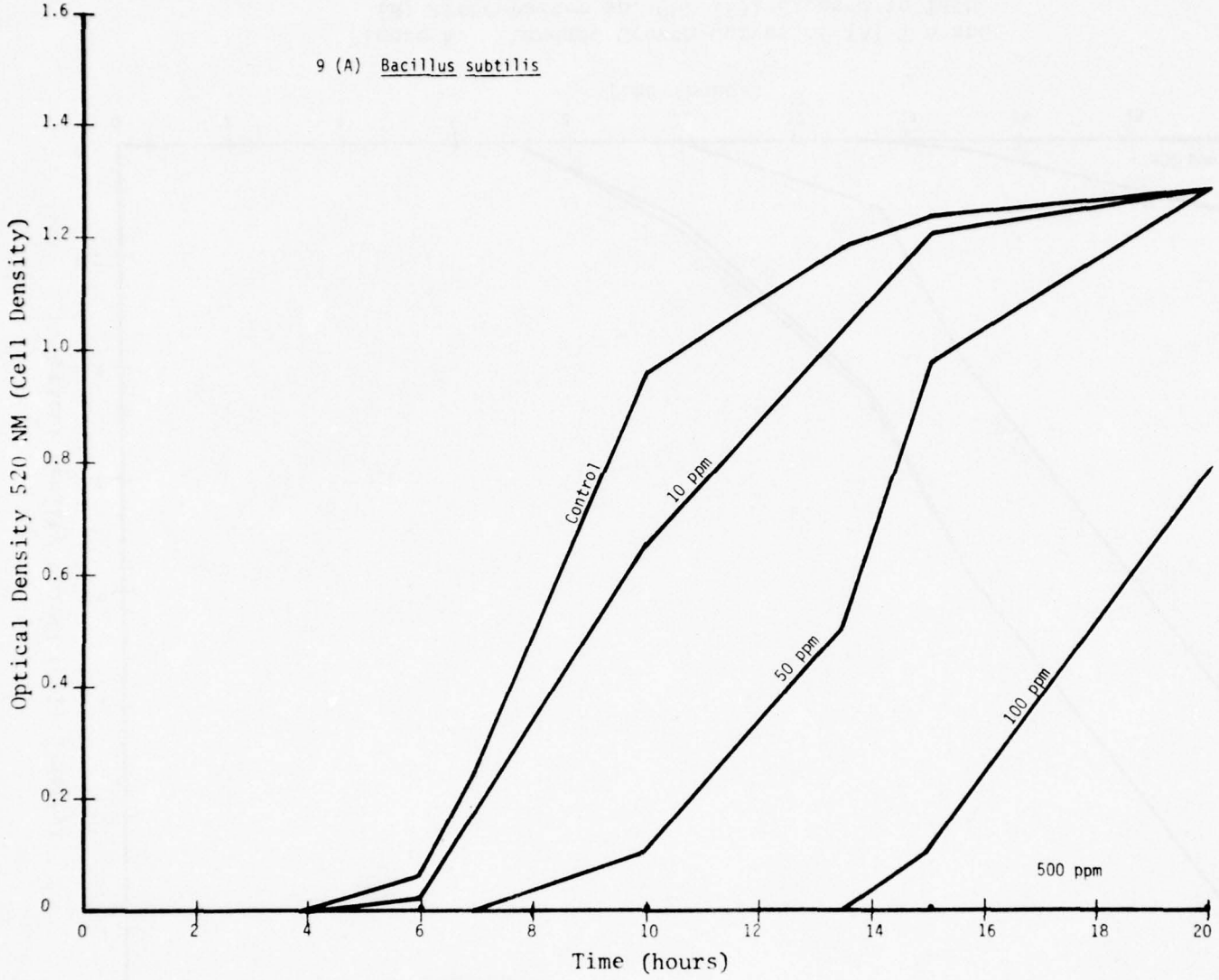


Figure 8. Standard Growth Curves of (A) T 6 and (B) Arthrobacter sp. QMB 1631 Exposed to TAGN


9 (A) Bacillus subtilis
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
Control
10 ppm
50 ppm
100 ppm
500 ppm

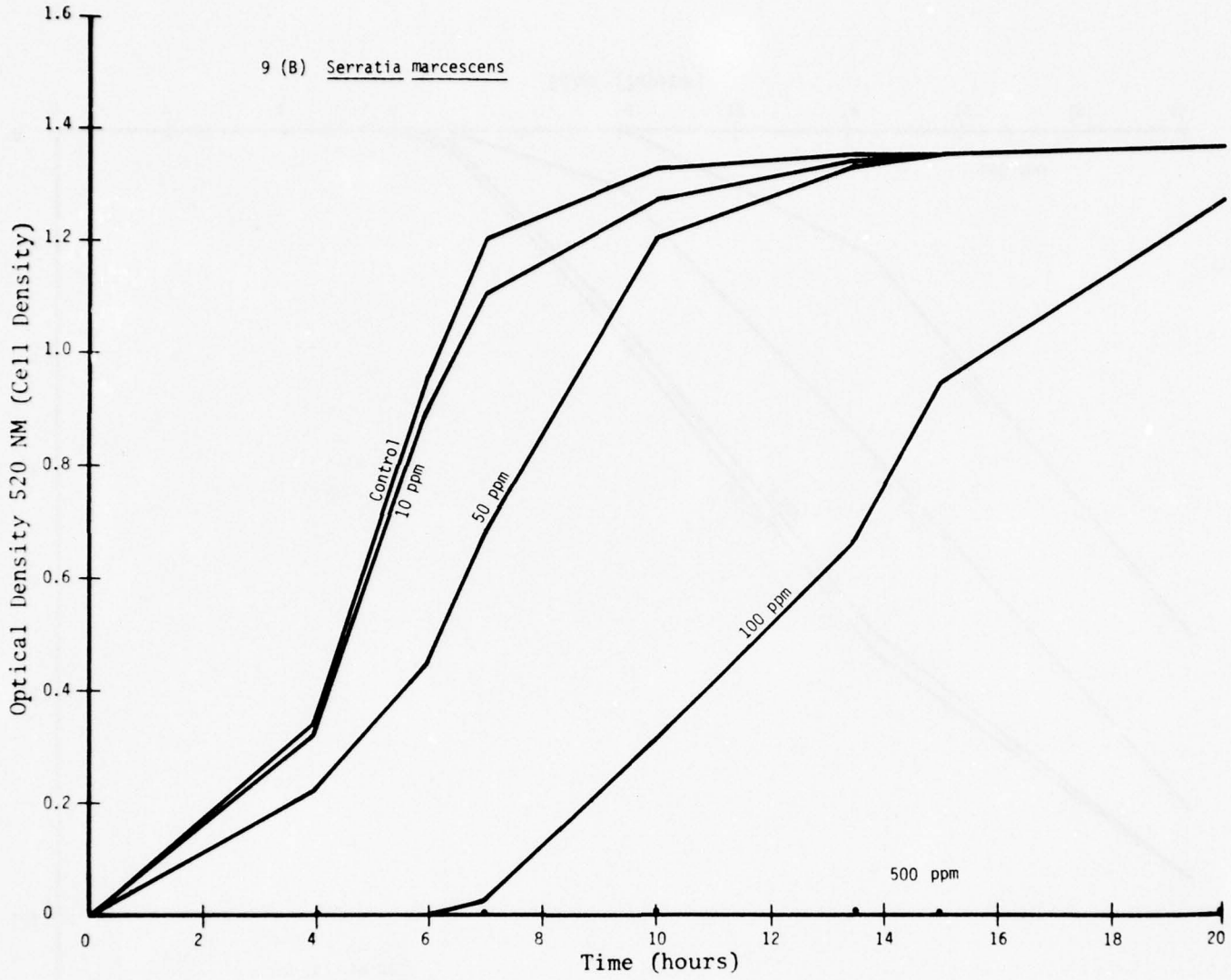
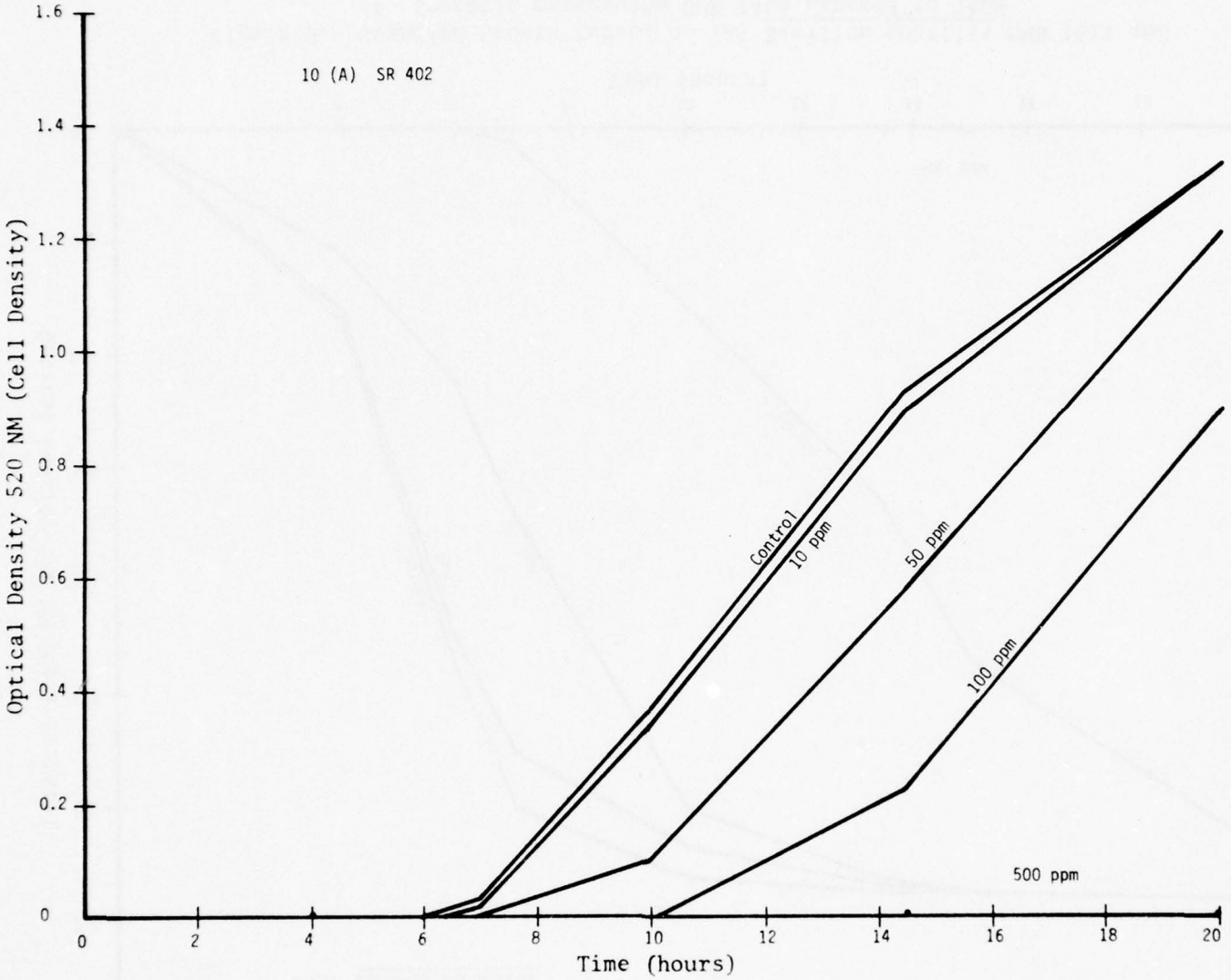


Figure 9. Standard Growth Curves of (A) Bacillus subtilis QMB 1611 and (B) Serratia marcescens QMB 1466 Exposed to TAGN


10 (A) SR 402
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
Control
10 ppm
50 ppm
100 ppm
500 ppm

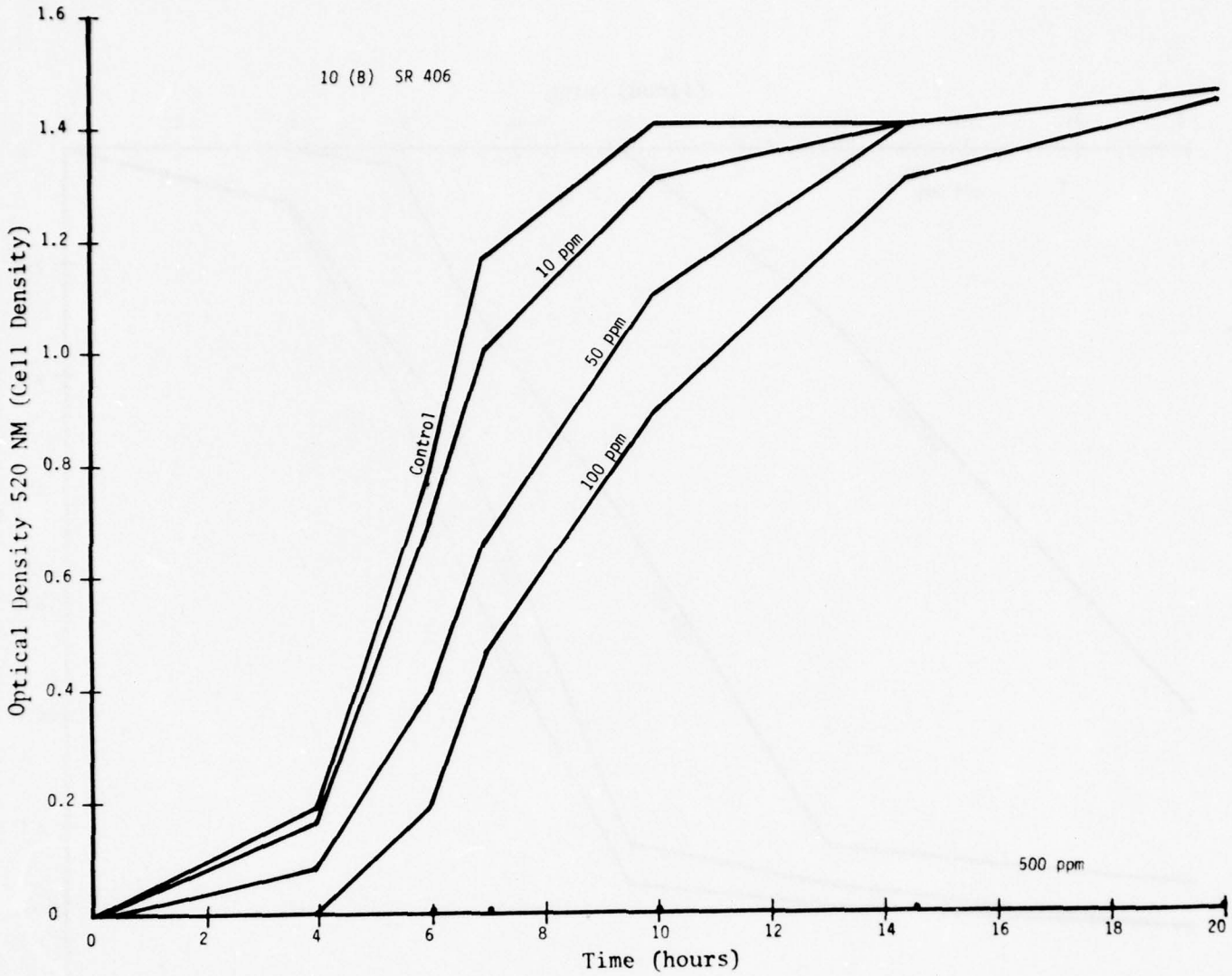
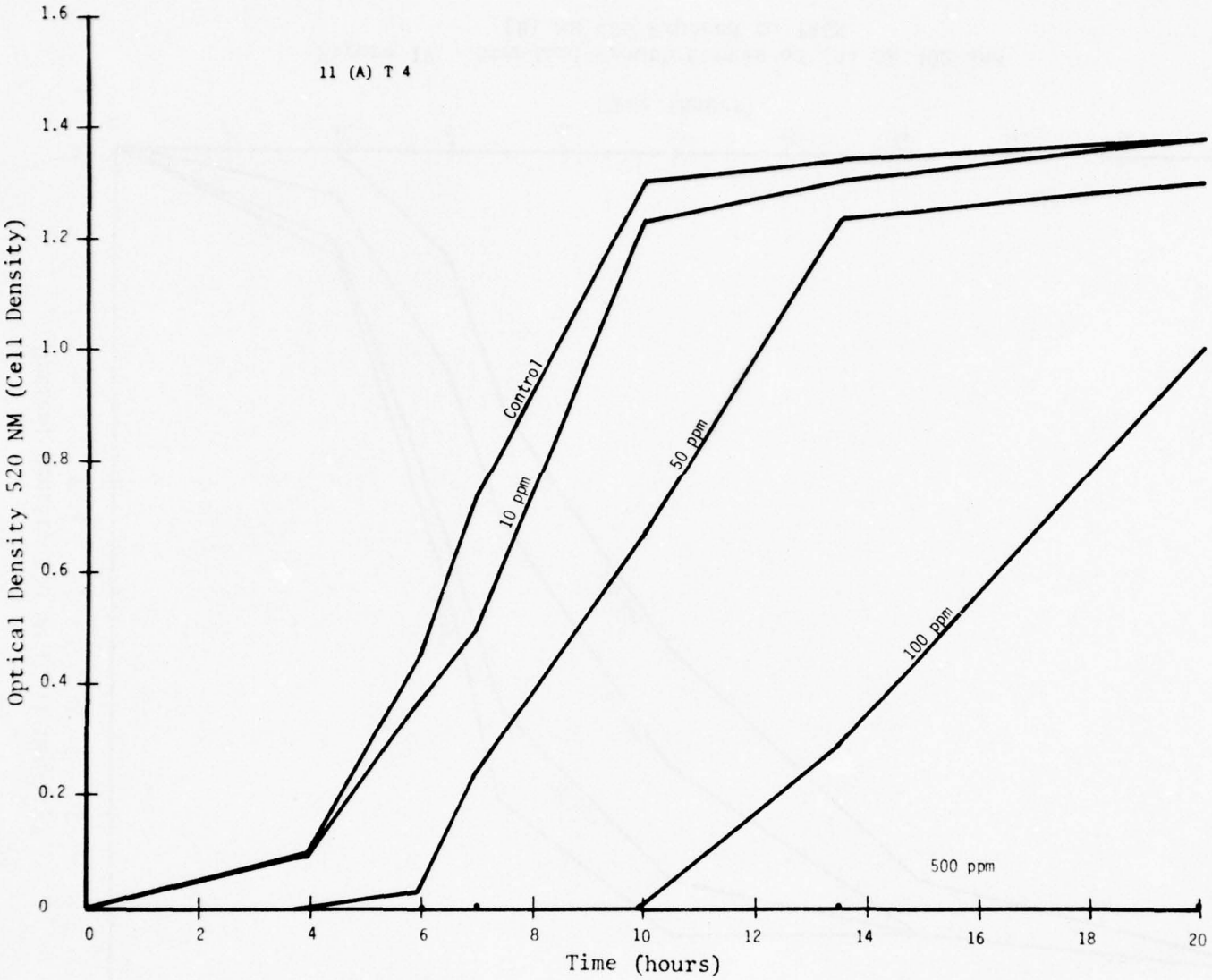


Figure 10. Standard Growth Curves of (A) SR 402 and (B) SR 406 Exposed to TAGN


11 (A) T 4
Optical Density 520 NM (Cell Density)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
18
20
Time (hours)
Control
10 ppm
50 ppm
100 ppm
500 ppm

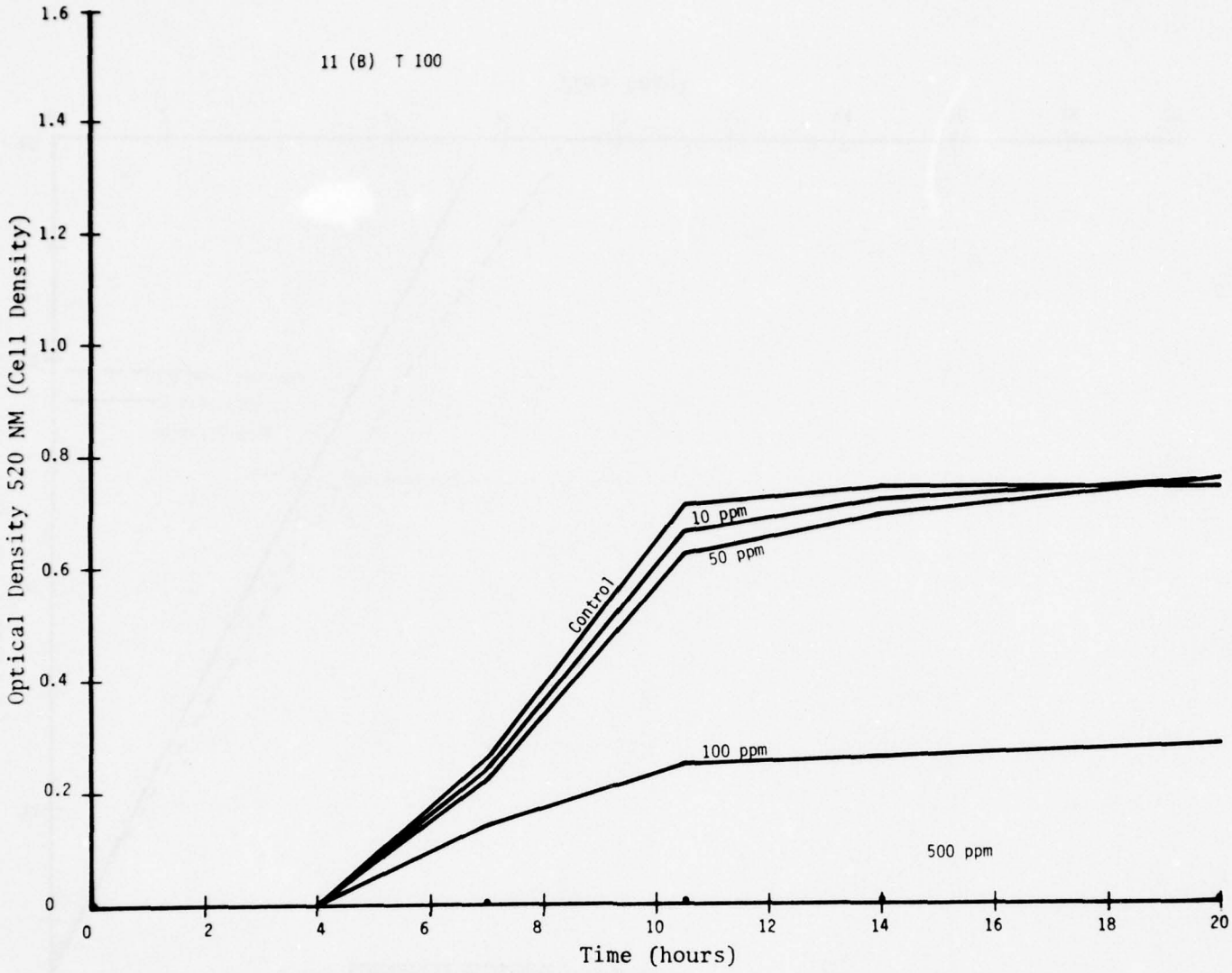
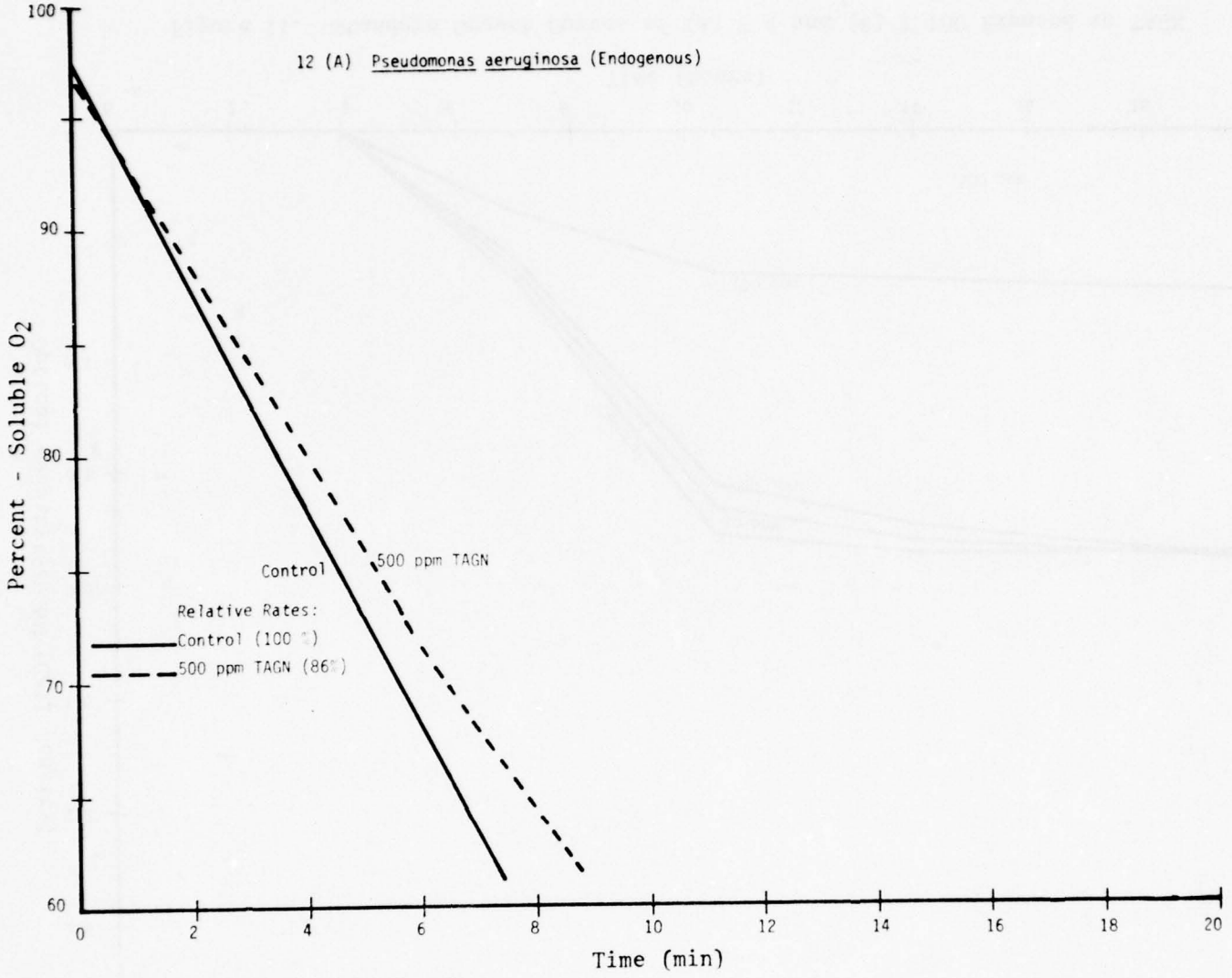


Figure 11. Standard Growth Curves of (A) T 4 and (B) T 100 Exposed to TAGN


12 (A) Pseudomonas aeruginosa (Endogenous)
Percent - Soluble O_2
100
90
80
70
60
0
2
4
6
8
10
12
14
16
18
20
Time (min)
Control
500 ppm TAGN
Relative Rates:
Control (100 %)
500 ppm TAGN (86%)

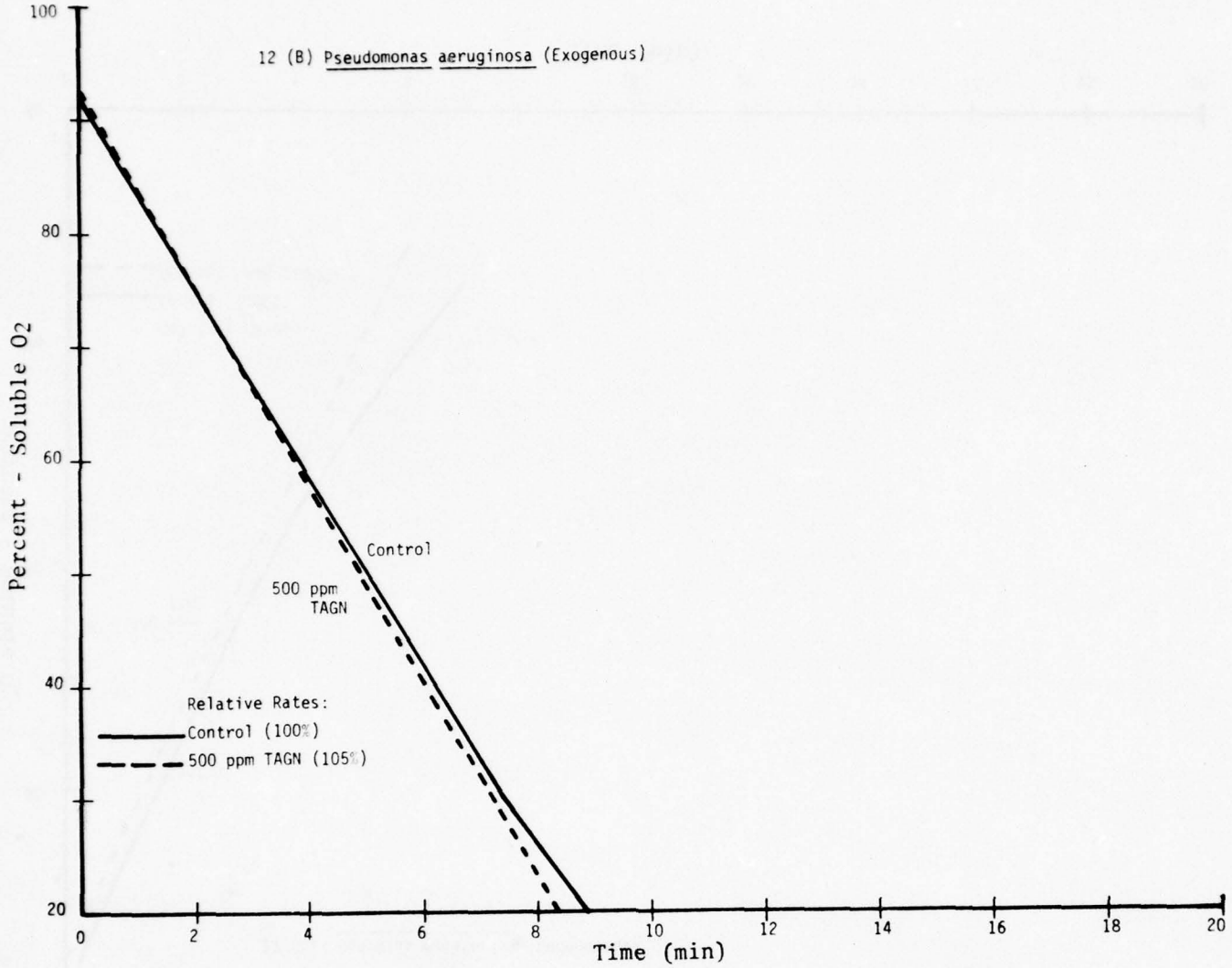
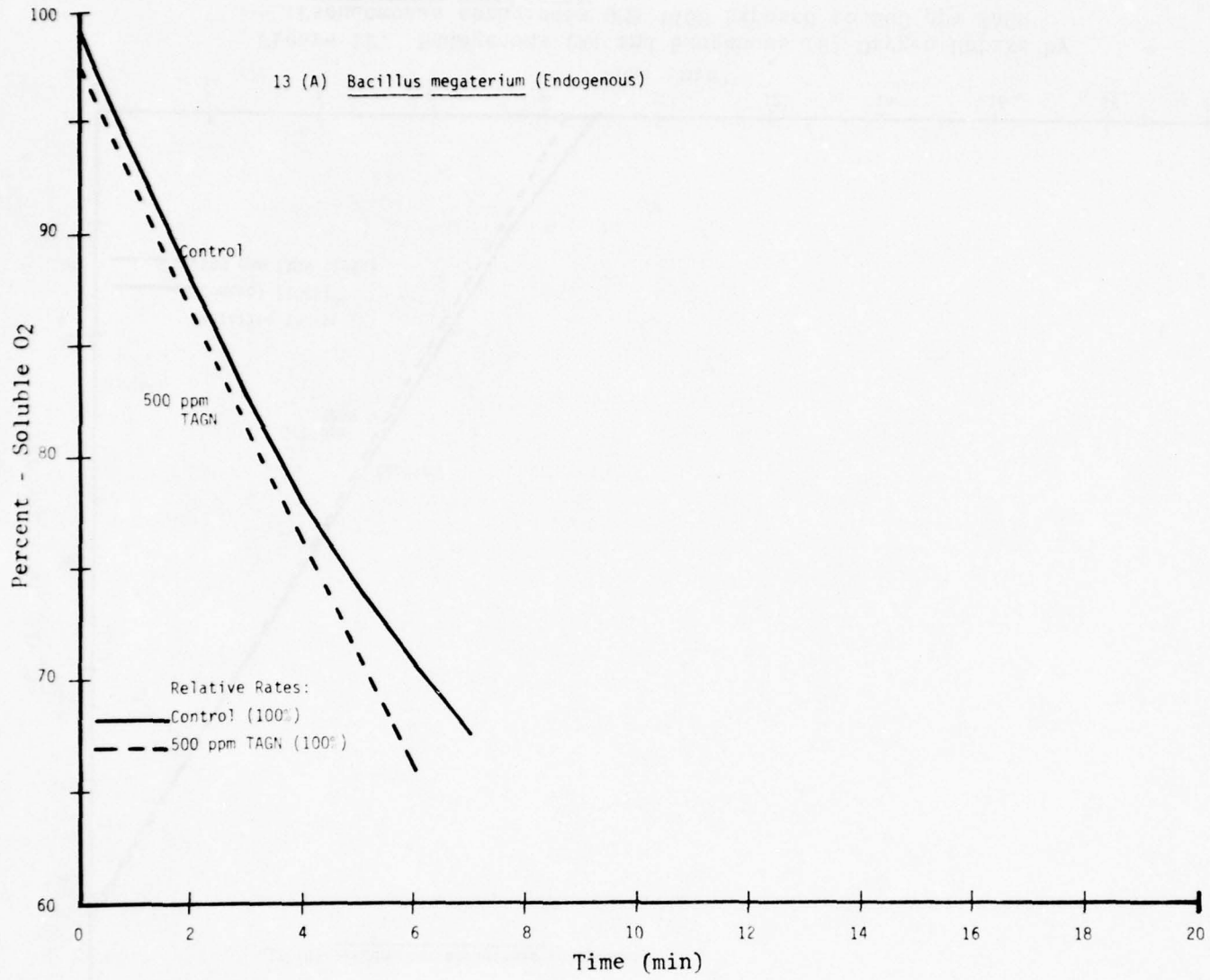


Figure 12. Endogenous (A) and Exogenous (B) Oxygen Uptake by Pseudomonas aeruginosa QMB 1468 Exposed to 500 ppm TAGN


13 (A) Bacillus megaterium (Endogenous)
Percent - Soluble O2
100
90
80
70
60
Control
500 ppm TAGN
Relative Rates:
Control (100%)
500 ppm TAGN (100%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

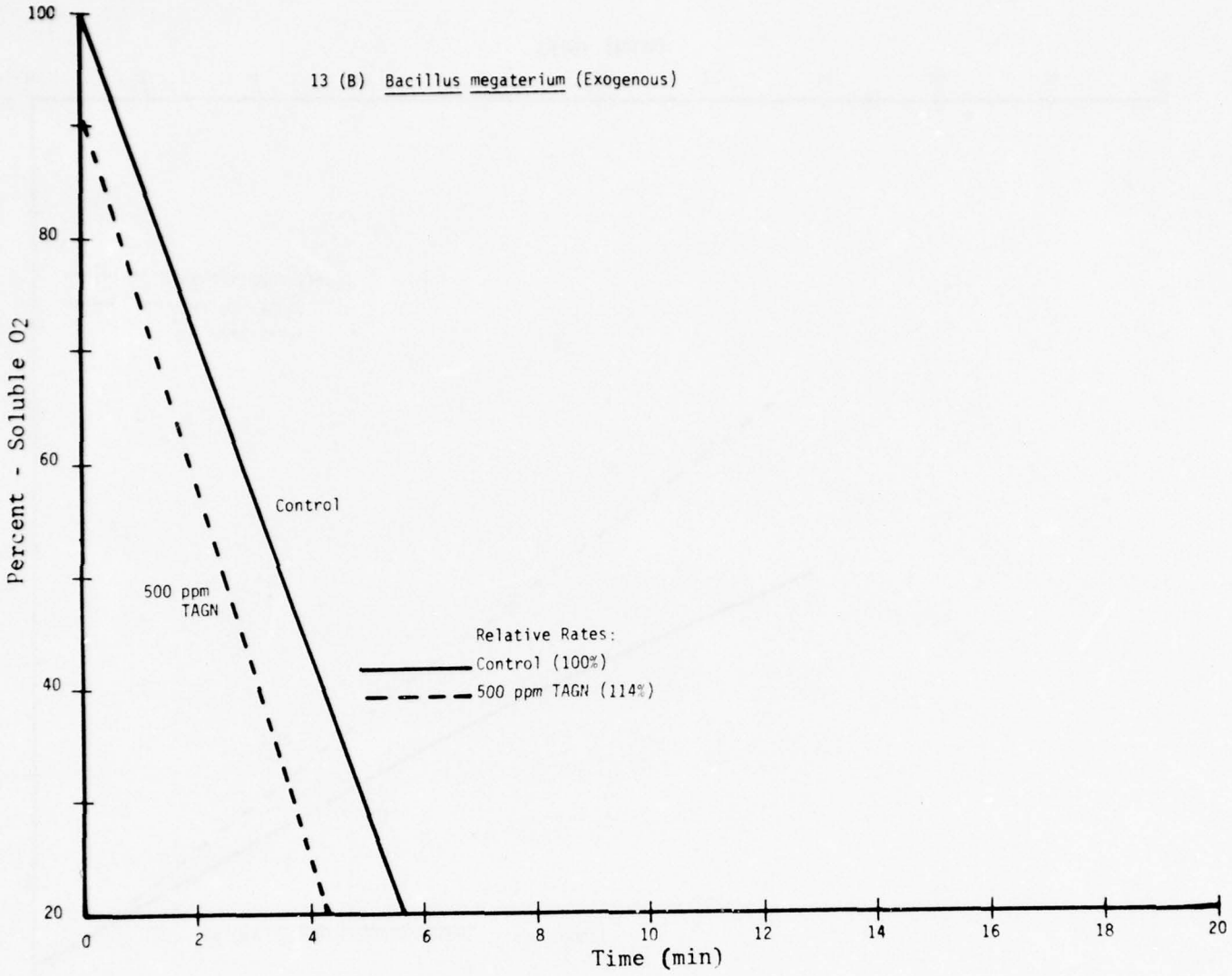
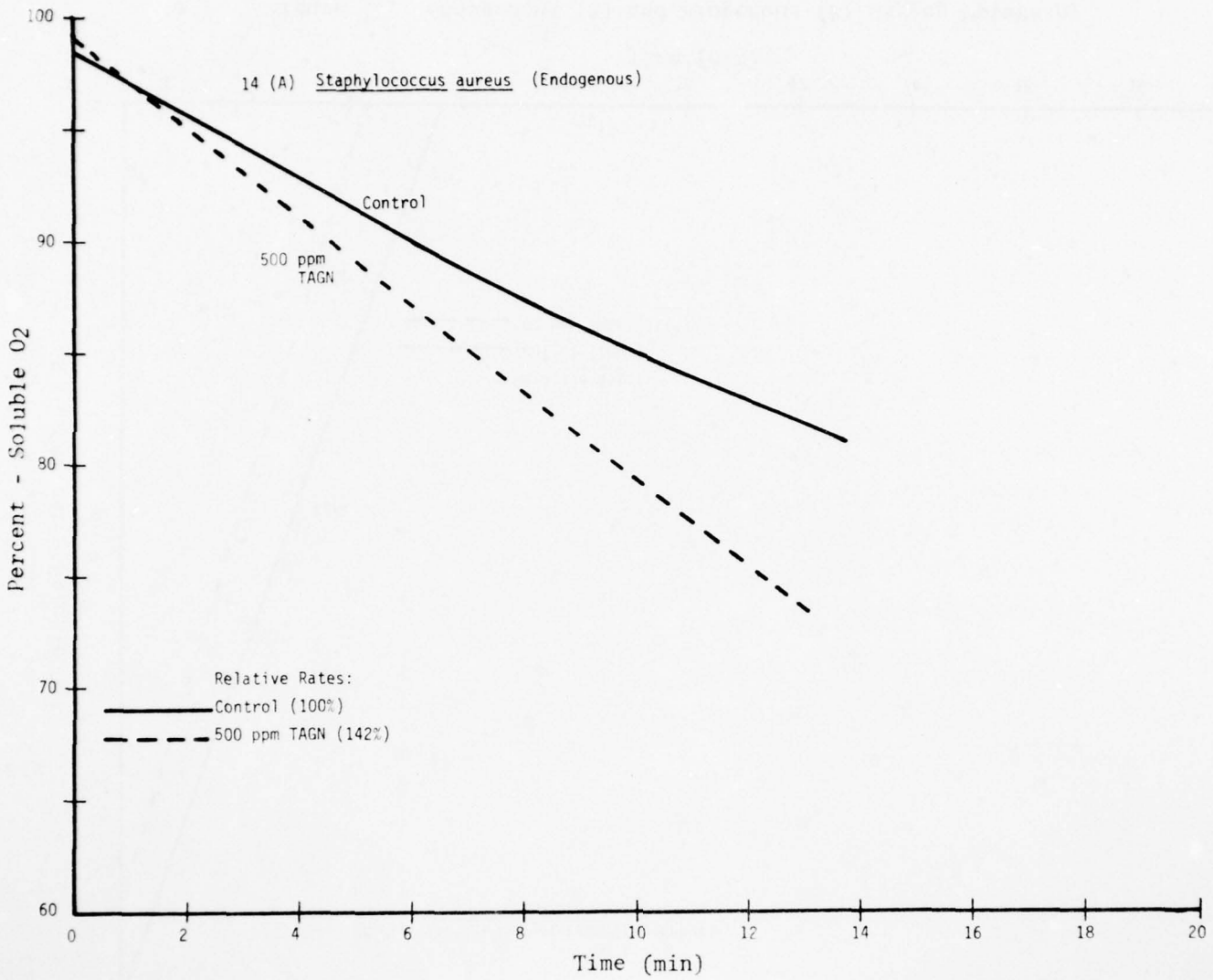


Figure 13. Endogenous (A) and Exogenous (B) Oxygen Uptake by Bacillus megaterium QMB 1605 Exposed to 500 ppm TAGN


14 (A) Staphylococcus aureus (Endogenous)
Control
500 ppm TAGN
Percent - Soluble O2
100
90
80
70
60
Relative Rates:
Control (100%)
500 ppm TAGN (142%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

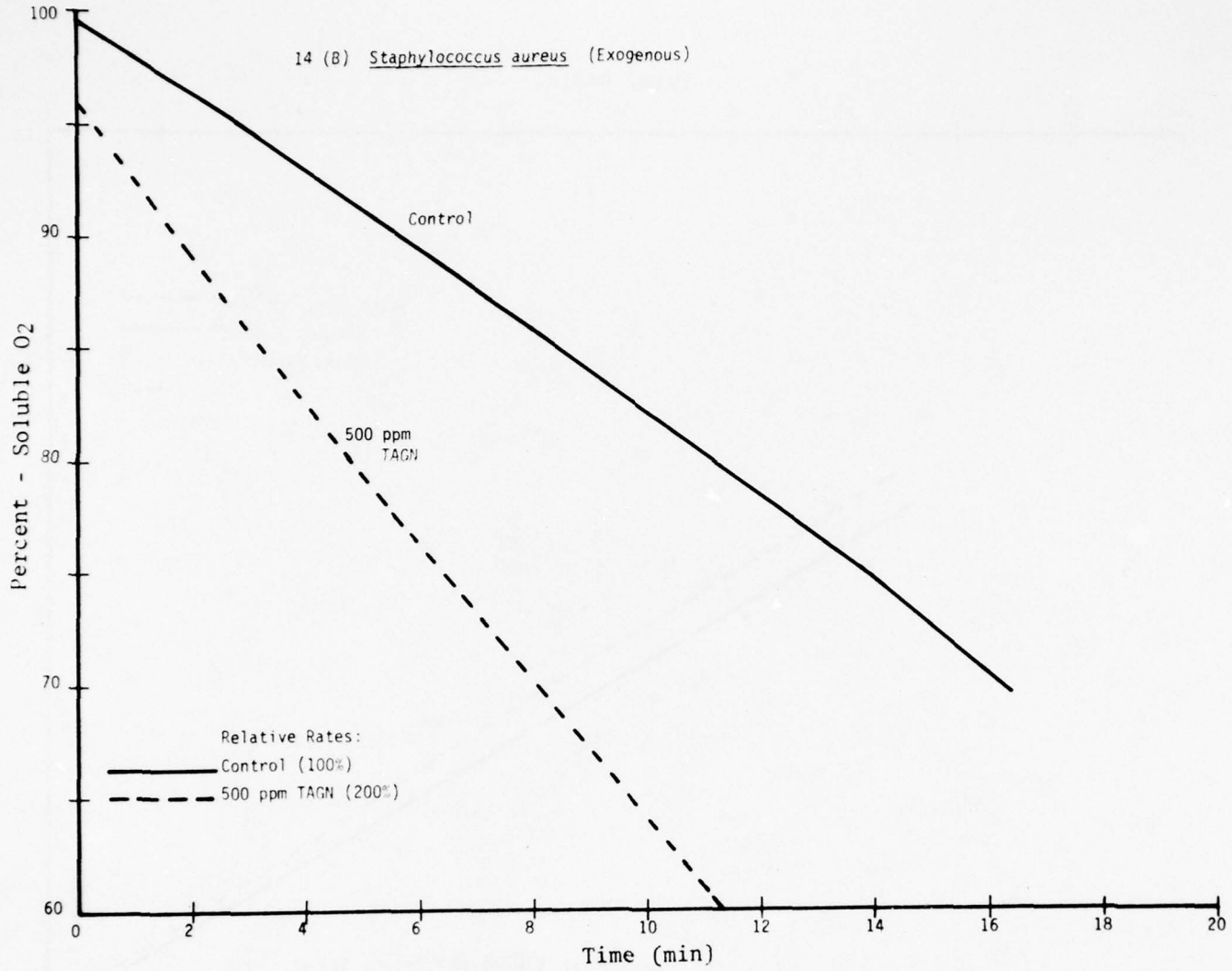
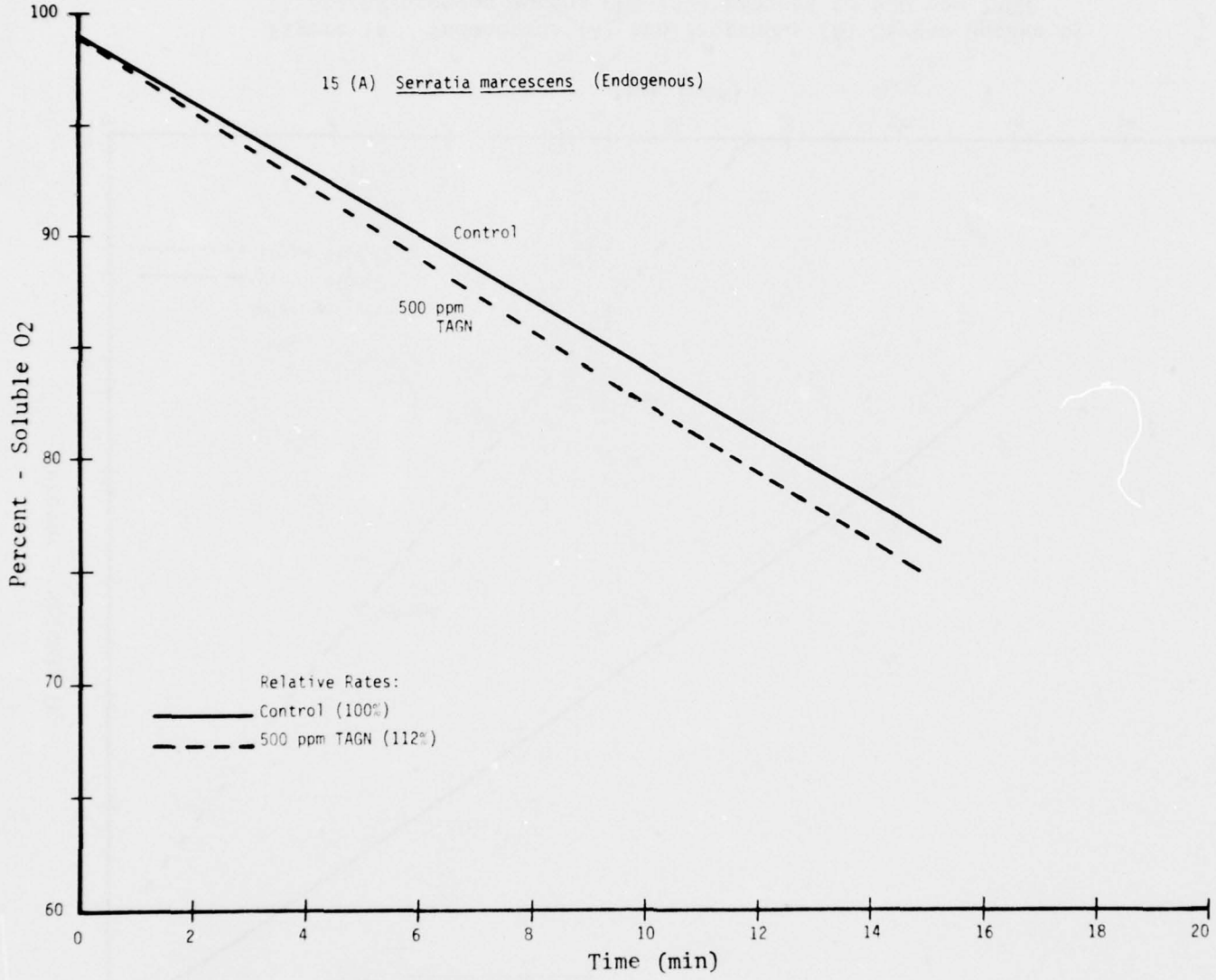


Figure 14. Endogenous (A) and Exogenous (B) Oxygen Uptake by Staphylococcus aureus QMB 1458 Exposed to 500 ppm TAGN


15 (A) Serratia marcescens (Endogenous)
Control
500 ppm TAGN
Relative Rates:
Control (100%)
500 ppm TAGN (112%)
Percent - Soluble O2
Time (min)
100
90
80
70
60
0
2
4
6
8
10
12
14
16
18
20

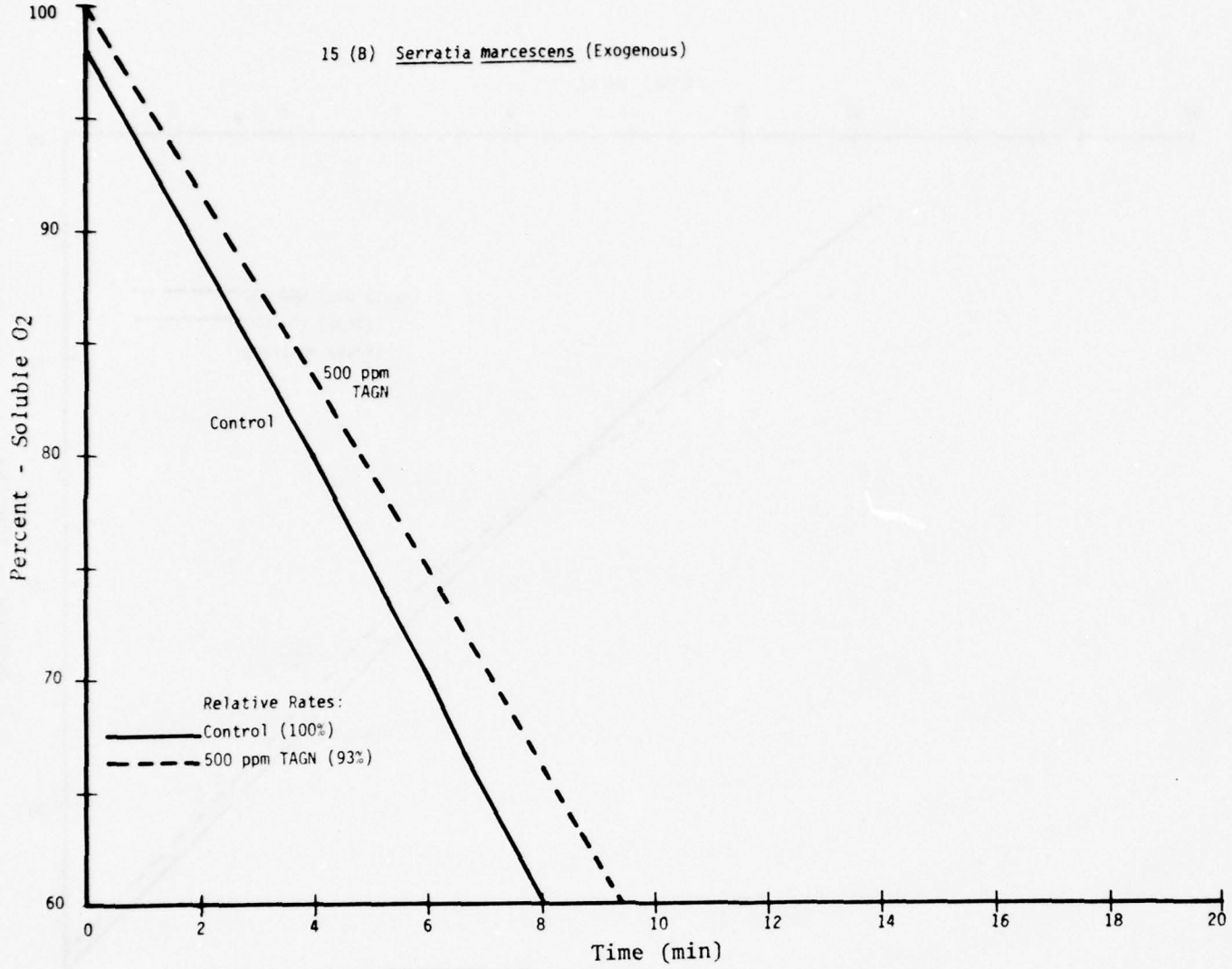
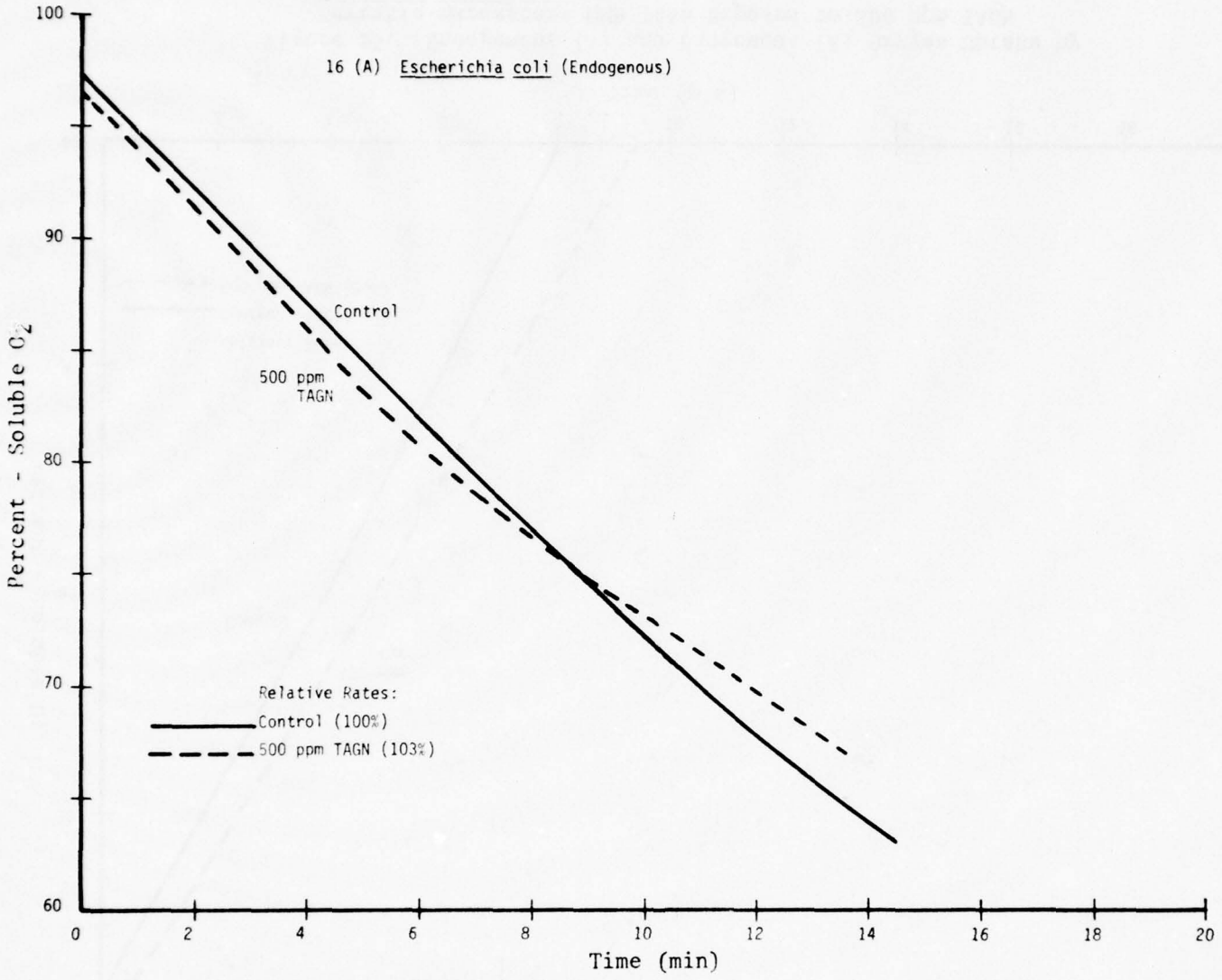


Figure 15. Endogenous (A) and Exogenous (B) Oxygen Uptake by Serratia marcescens QMB 1466 Exposed to 500 ppm TAGN


16 (A) Escherichia coli (Endogenous)
Percent - Soluble O2
100
90
80
70
60
Control
500 ppm TAGN
Relative Rates:
Control (100%)
500 ppm TAGN (103%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

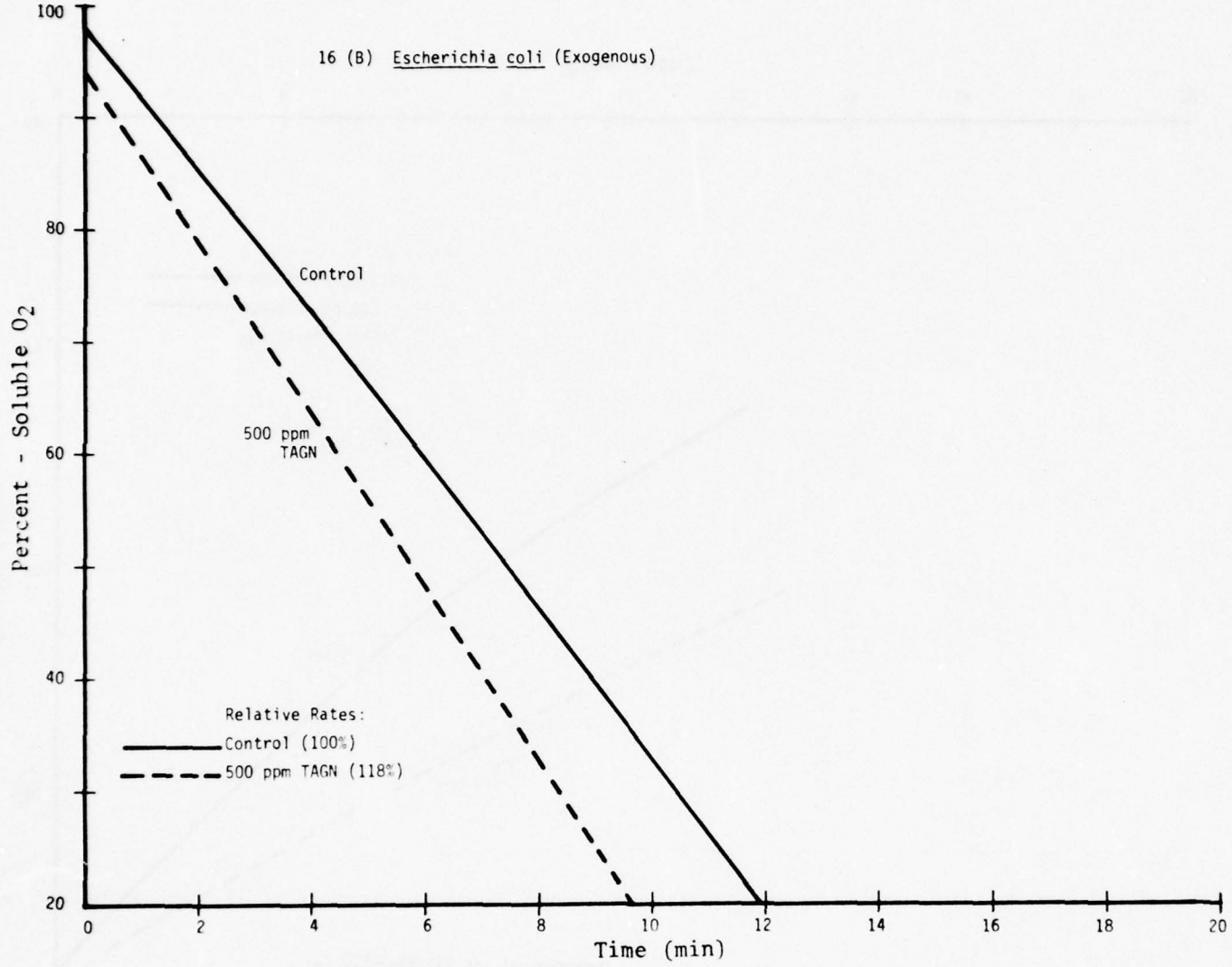
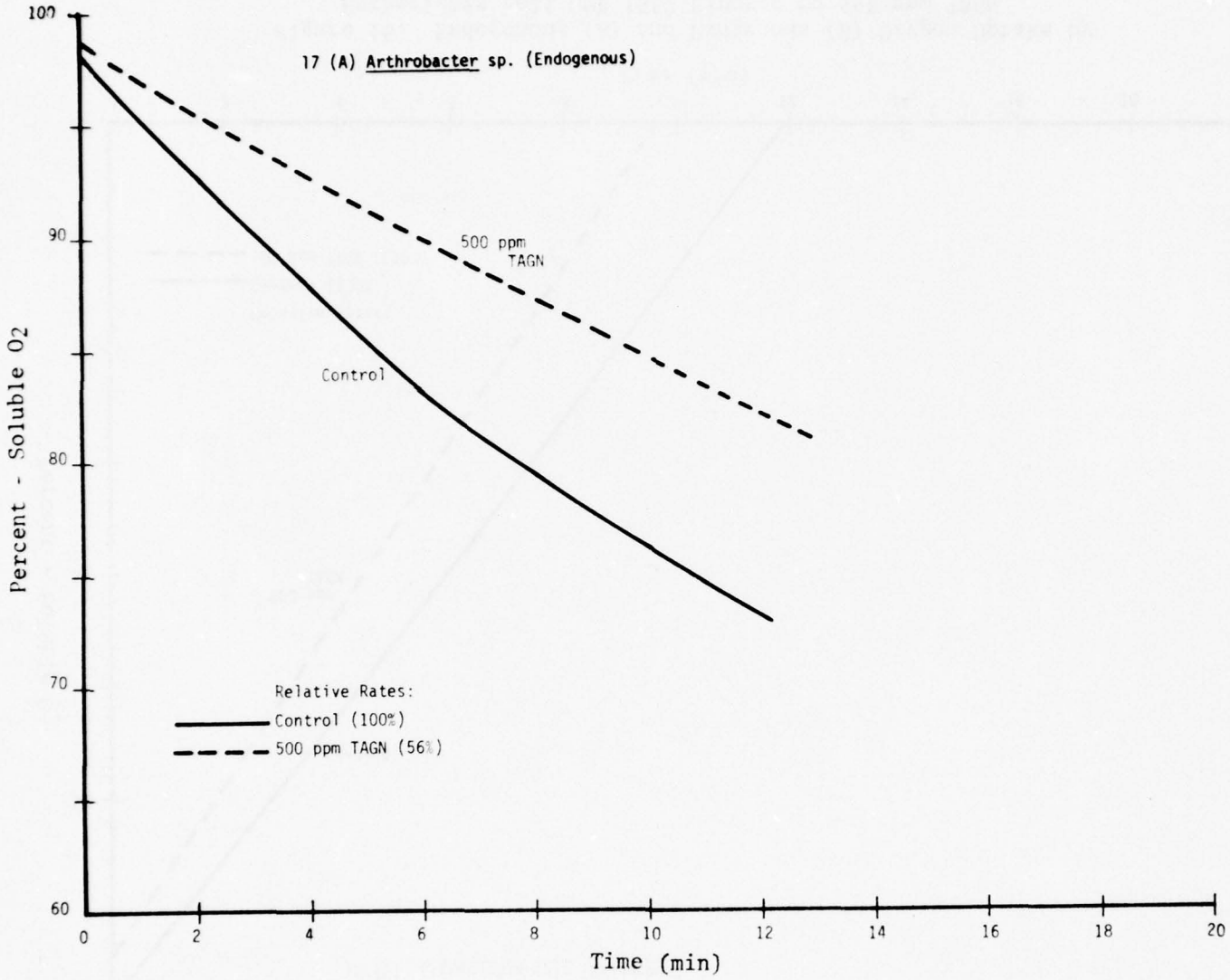


Figure 16. Endogenous (A) and Exogenous (B) Oxygen Uptake by *Escherichia coli* QMB 1557 Exposed to 500 ppm TAGN


17 (A) Arthrobacter sp. (Endogenous)
100
90
80
70
60
Percent - Soluble O2
500 ppm
TAGN
Control
Relative Rates:
Control (100%)
500 ppm TAGN (56%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

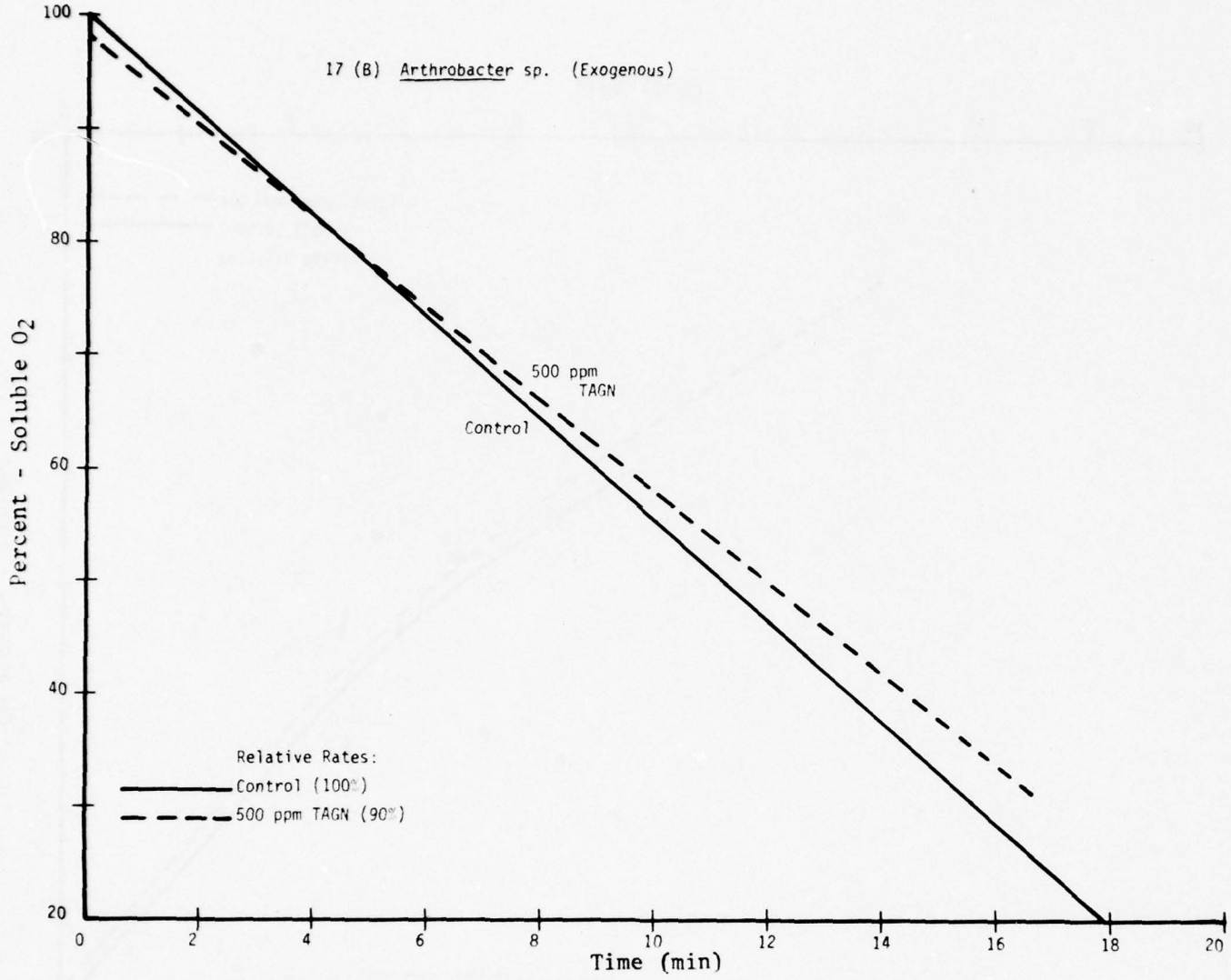
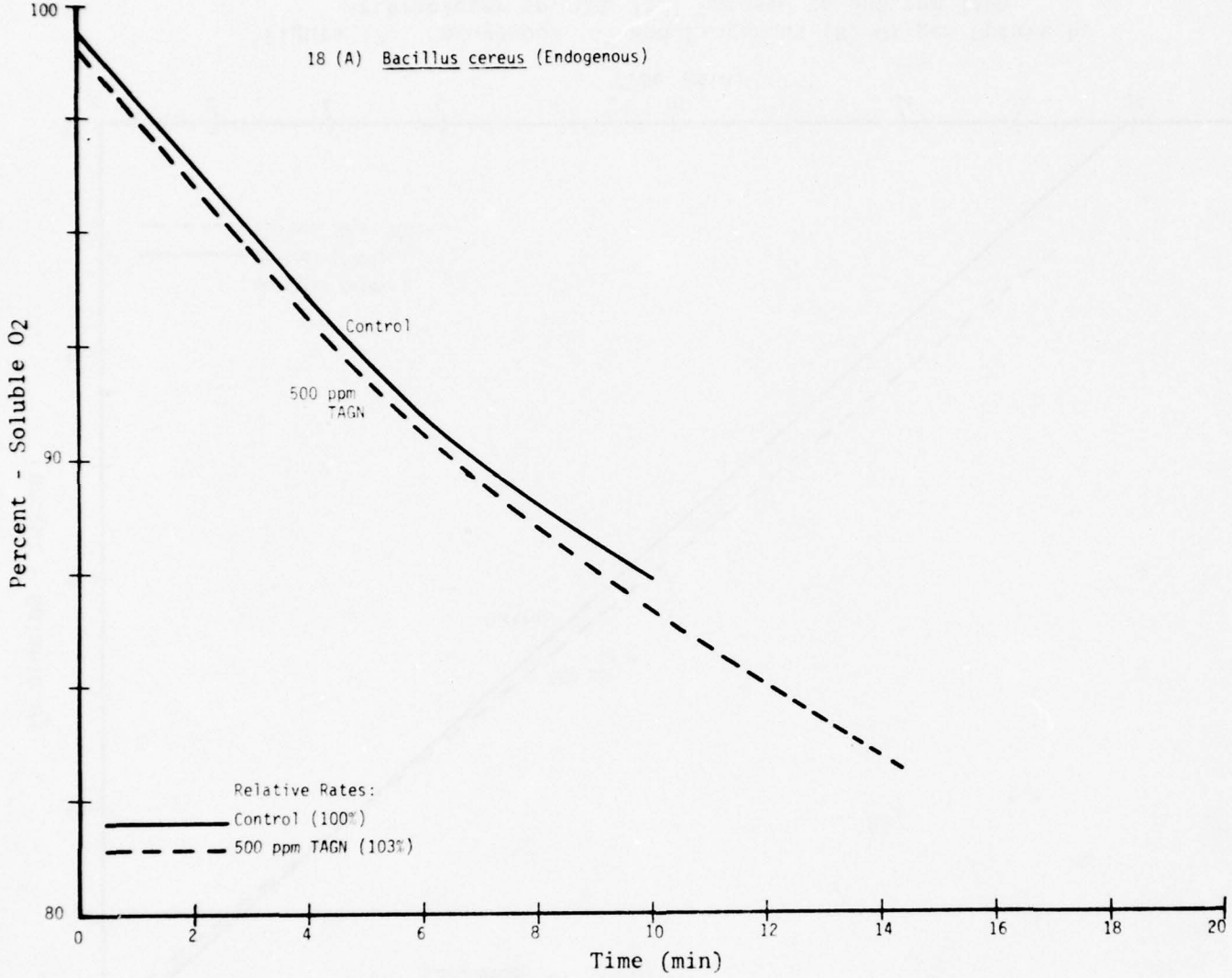


Figure 17. Endogenous (A) and Exogenous (B) Oxygen Uptake by Arthrobacter sp. QMB 1631 Exposed to 500 ppm TAGN


18 (A) Bacillus cereus (Endogenous)
100
90
80
Percent - Soluble O2
Control
500 ppm TAGN
Relative Rates:
Control (100%)
500 ppm TAGN (103%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

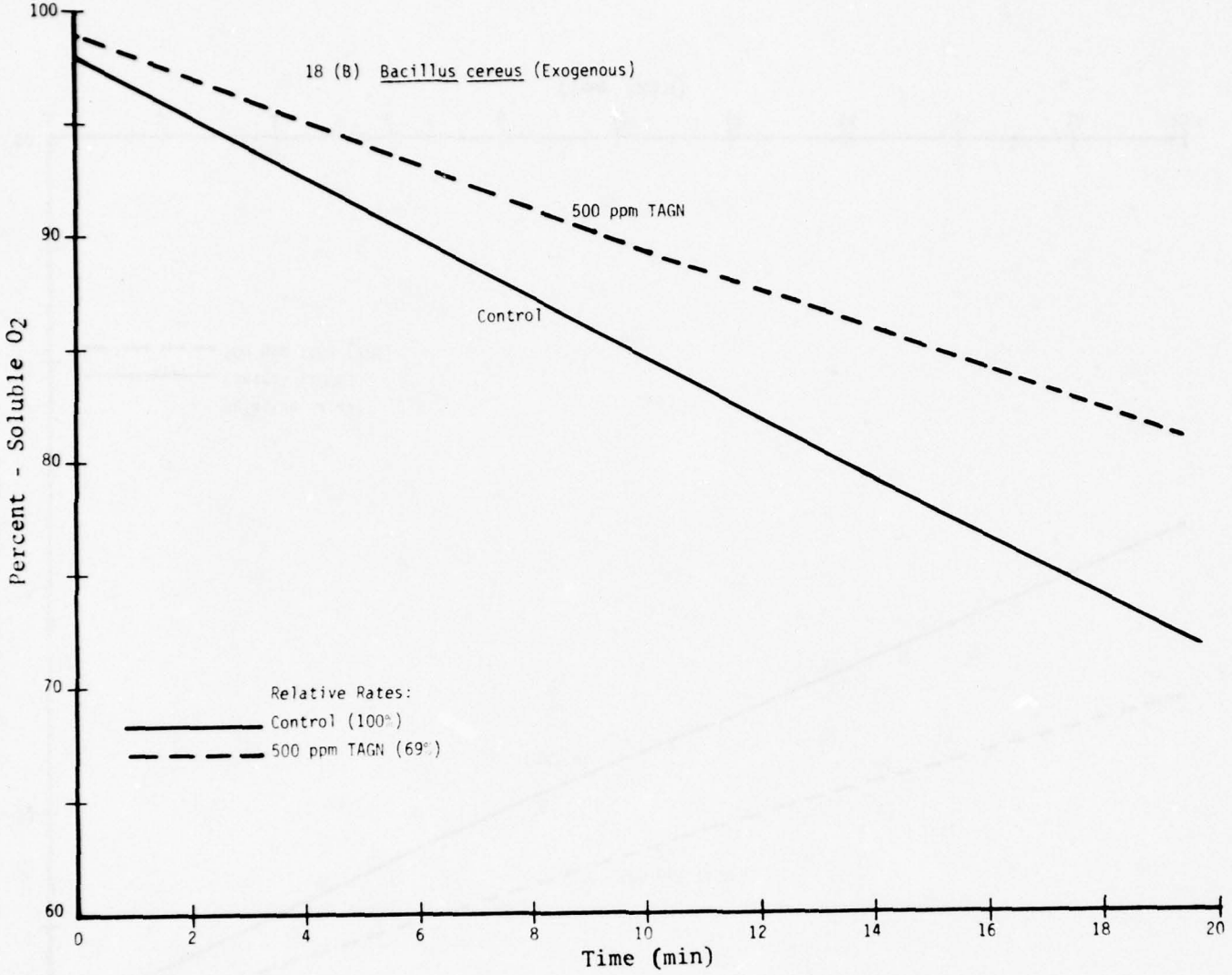
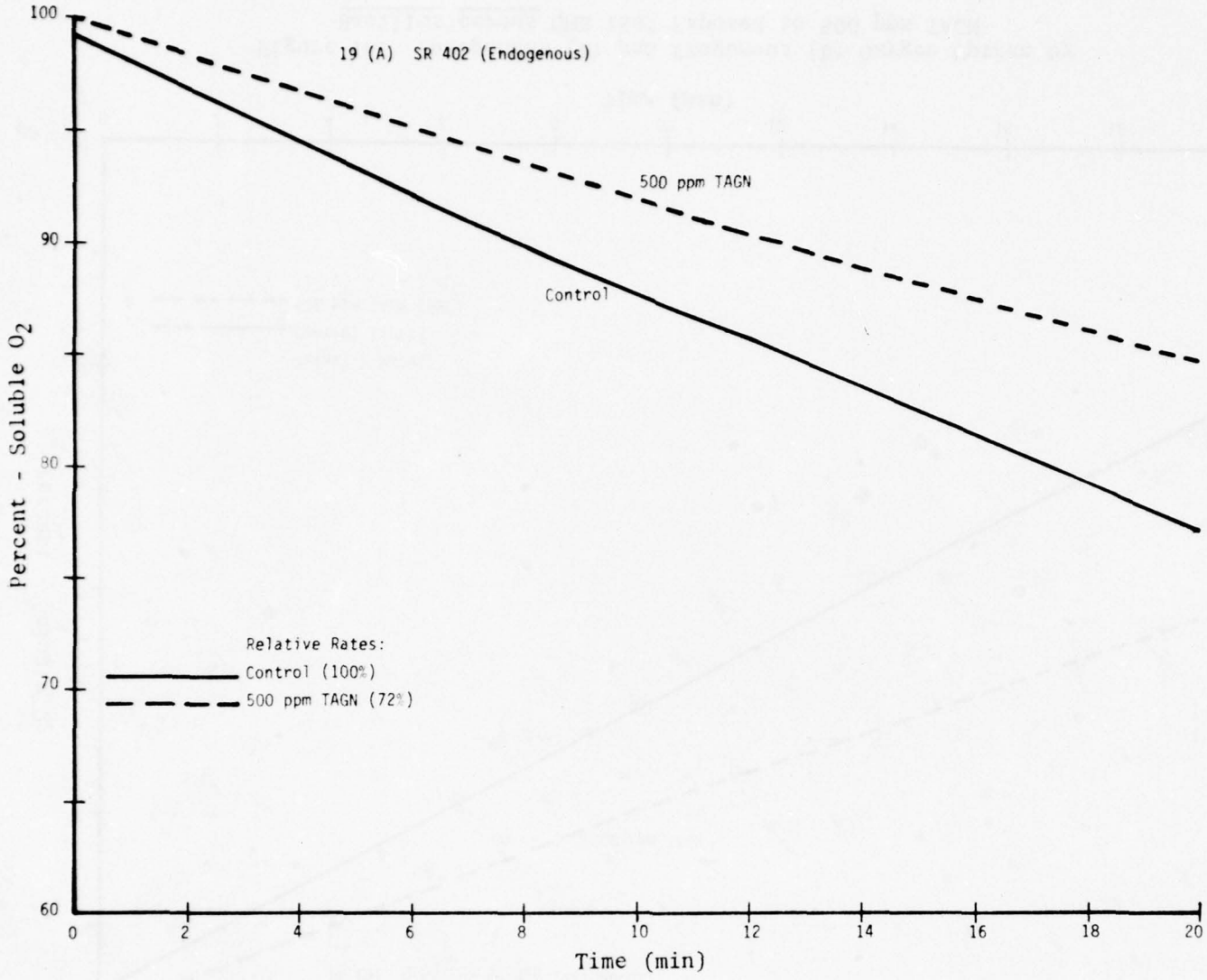


Figure 18. Endogenous (A) and Exogenous (B) Oxygen Uptake by Bacillus cereus QMB 1597 Exposed to 500 ppm TAGN


19 (A) SR 402 (Endogenous)
500 ppm TAGN
Control
Percent - Soluble O_2
100
90
80
70
60
0
2
4
6
8
10
12
14
16
18
20
Time (min)
Relative Rates:
Control (100%)
500 ppm TAGN (72%)

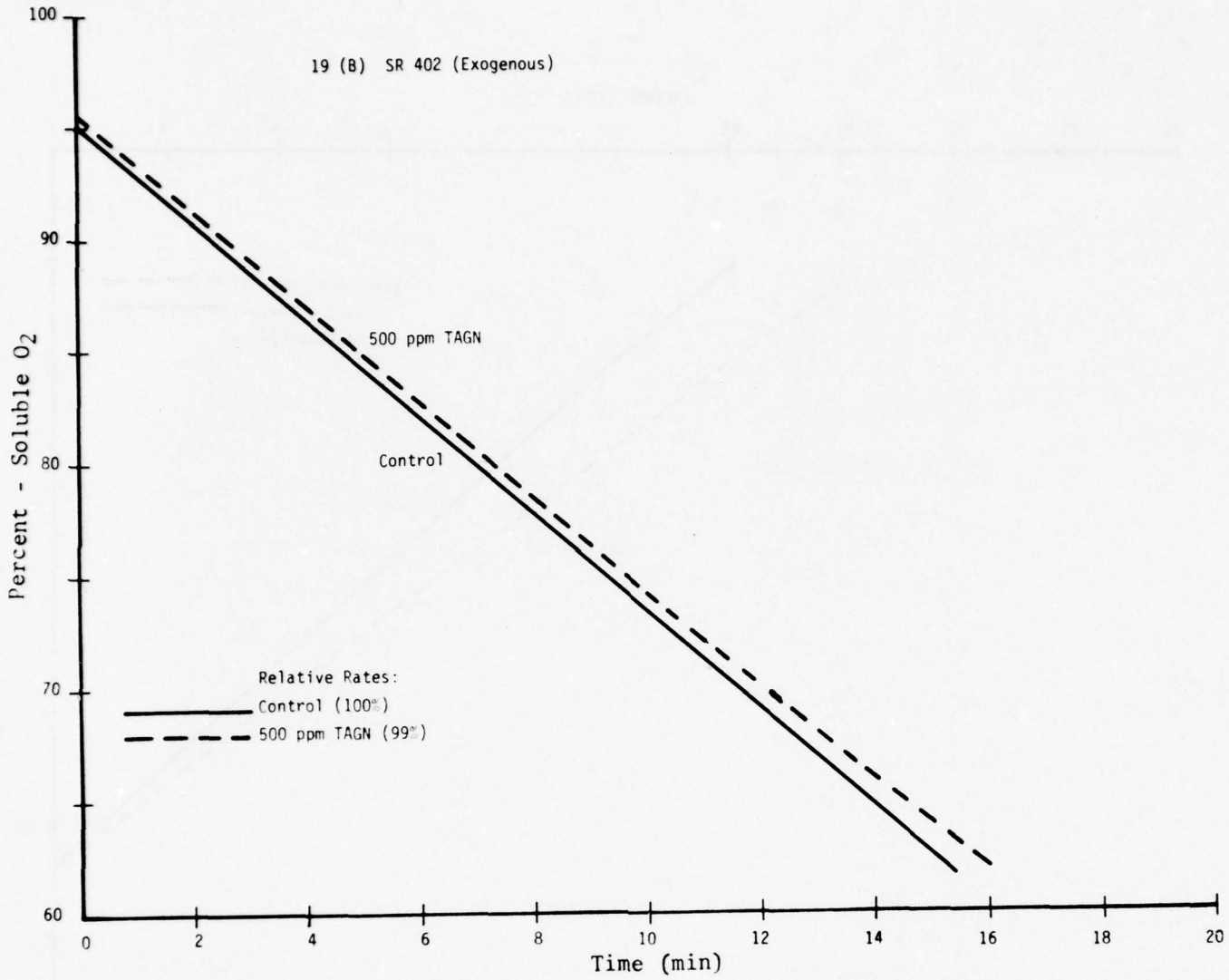
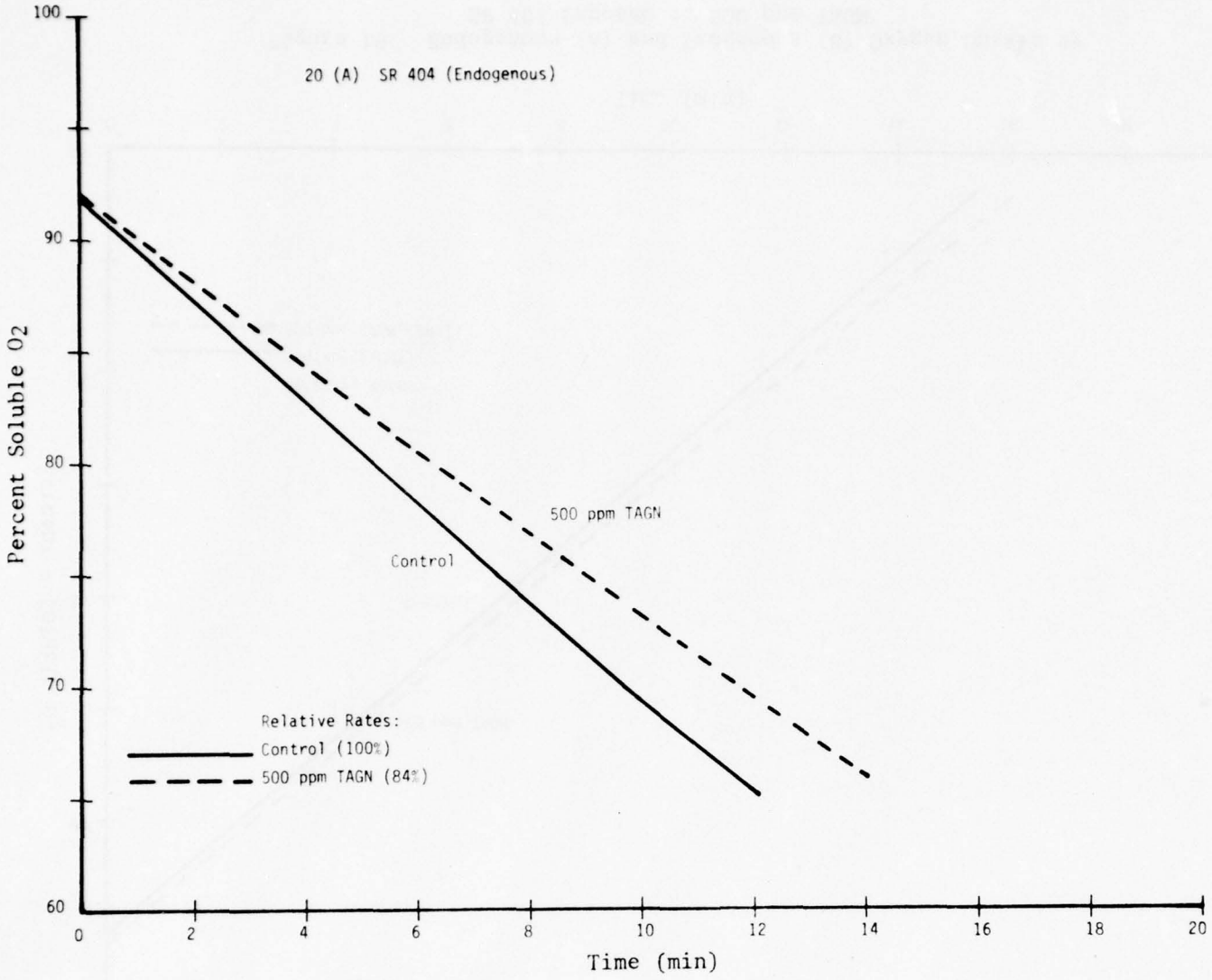


Figure 19. Endogenous (A) and Exogenous (B) Oxygen Uptake by SR 402 Exposed to 500 ppm TAGN


20 (A) SR 404 (Endogenous)
Percent Soluble O2
100
90
80
70
60
0
2
4
6
8
10
12
14
16
18
20
Time (min)
500 ppm TAGN
Control
Relative Rates:
Control (100%)
500 ppm TAGN (84%)

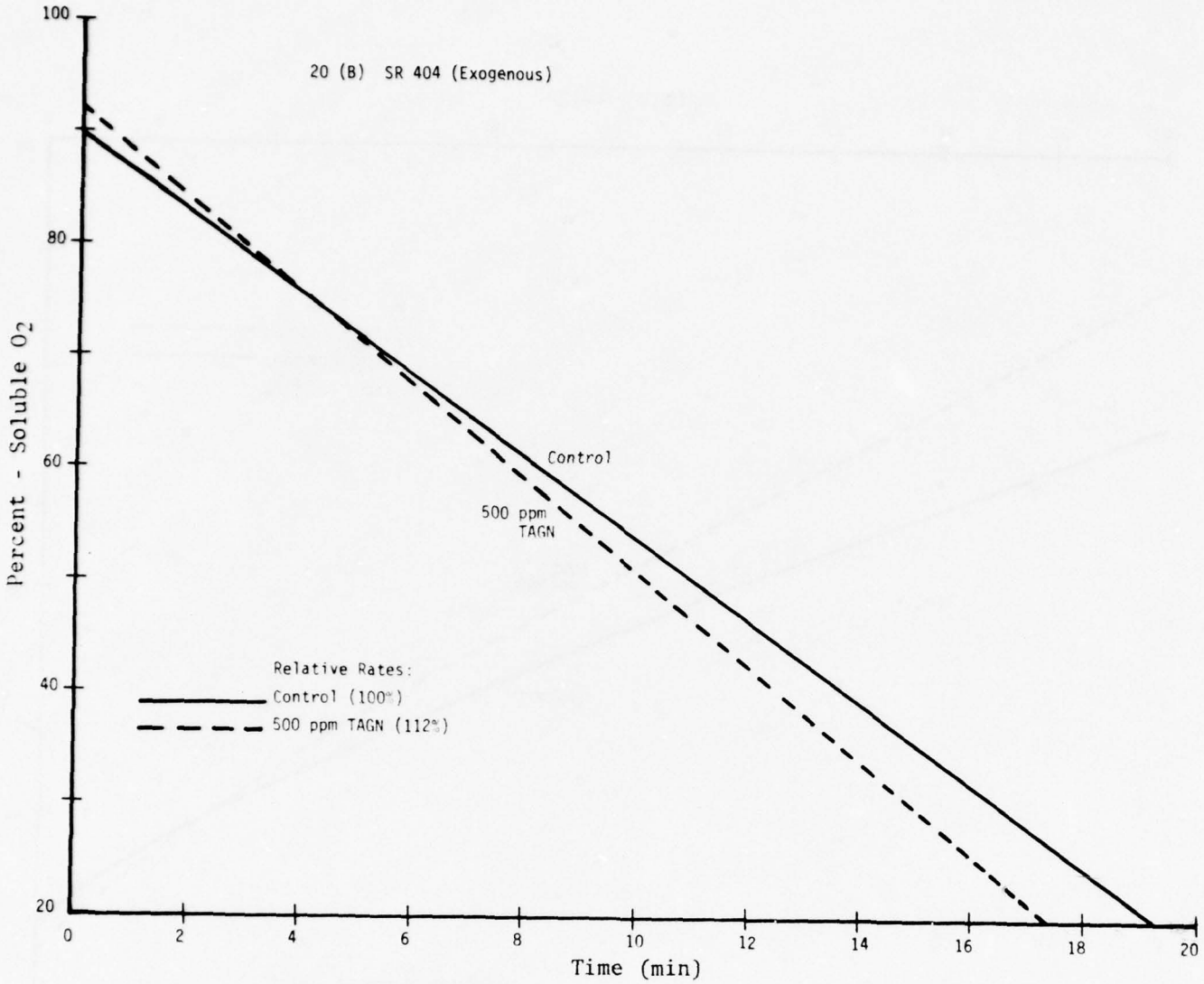
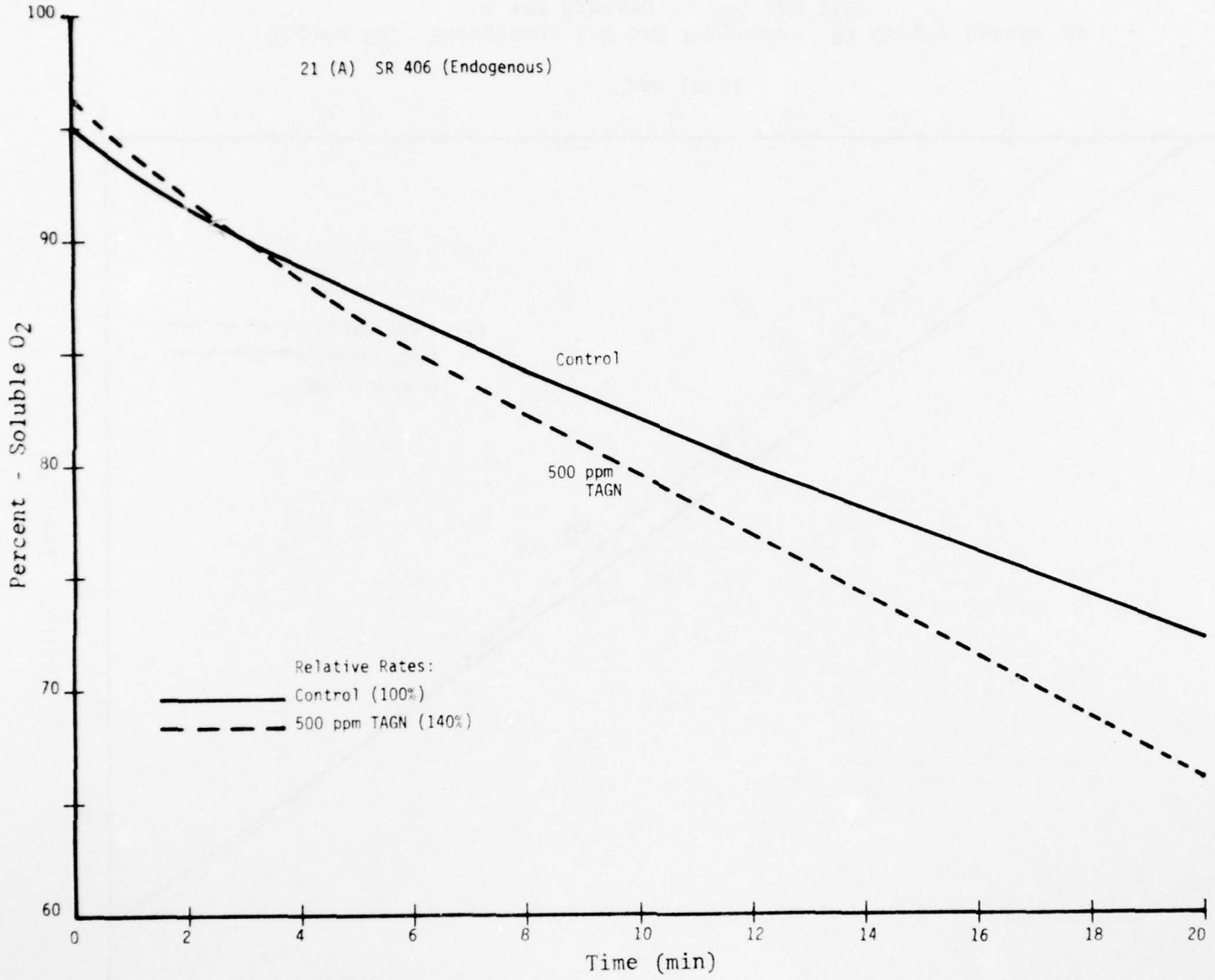


Figure 20. Endogenous (A) and Exogenous (B) Oxygen Uptake by SR 404 Exposed to 500 ppm TAGN


21 (A) SR 406 (Endogenous)
Percent - Soluble O_2
100
90
80
70
60
Control
500 ppm TAGN
Relative Rates:
Control (100%)
500 ppm TAGN (140%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

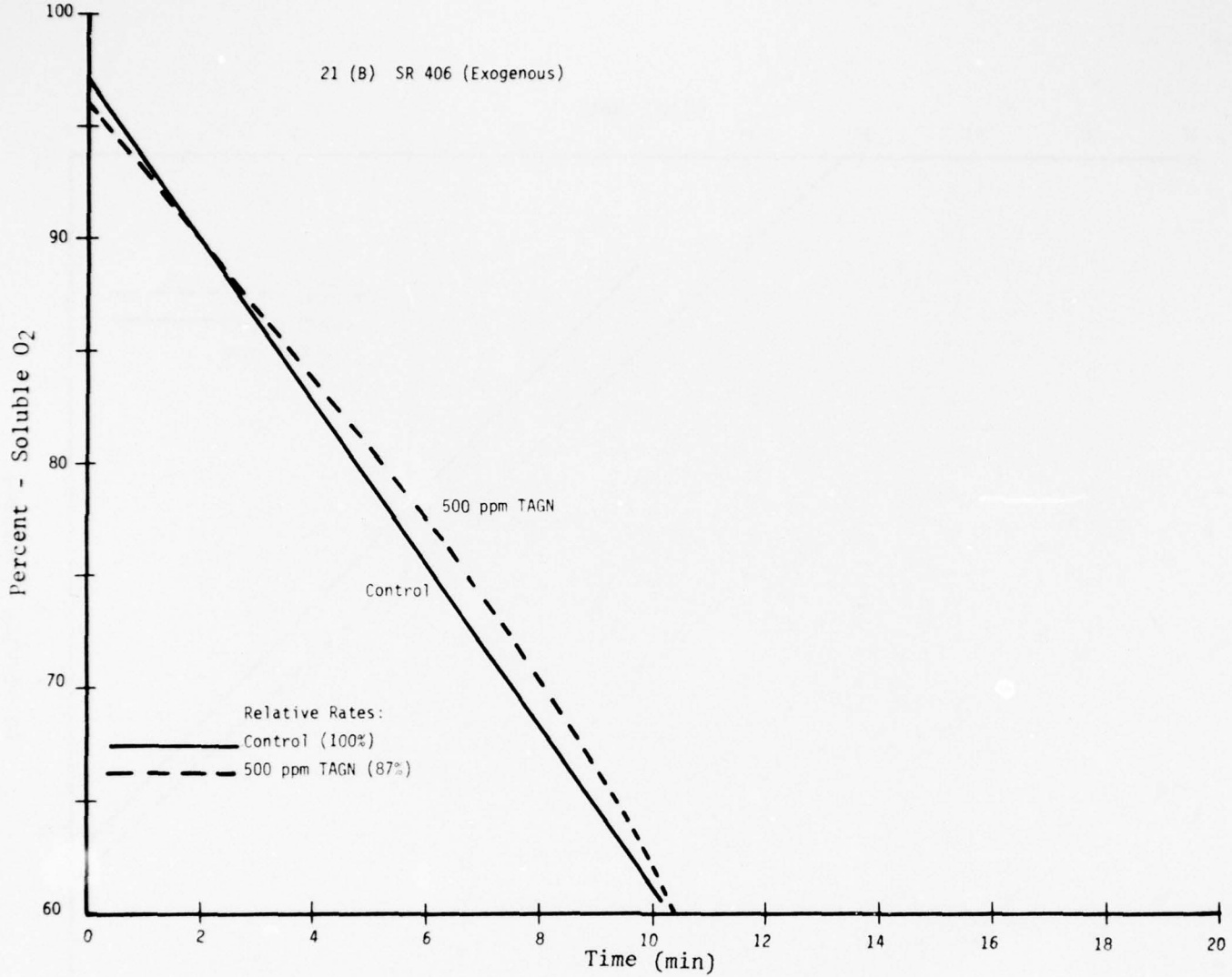
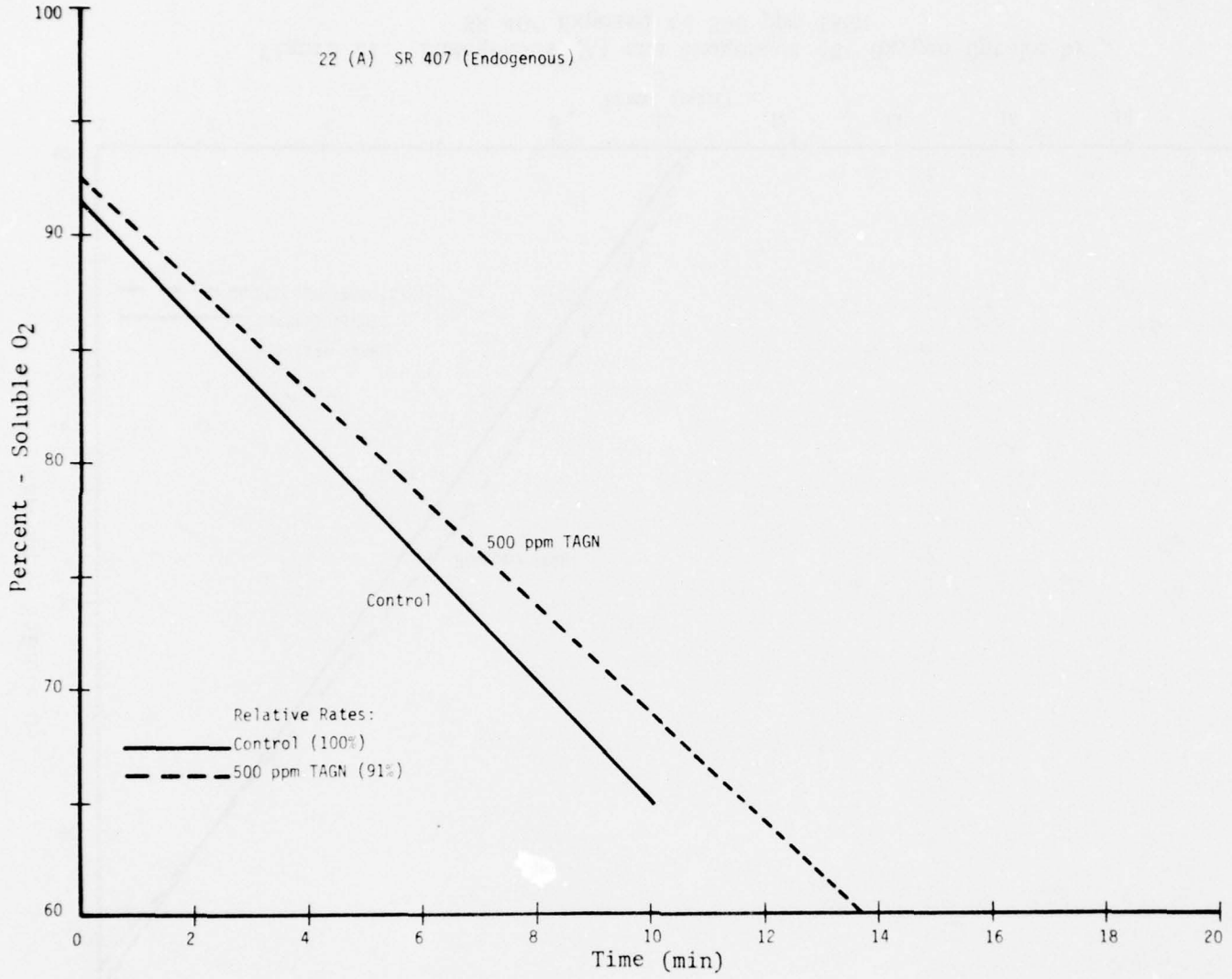


Figure 21. Endogenous (A) and Exogenous (B) Oxygen Uptake by SR 406 Exposed to 500 ppm TAGN


22 (A) SR 407 (Endogenous)
Percent - Soluble O_2
100
90
80
70
60
0
2
4
6
8
10
12
14
16
18
20
Time (min)
500 ppm TAGN
Control
Relative Rates:
Control (100%)
500 ppm TAGN (91%)

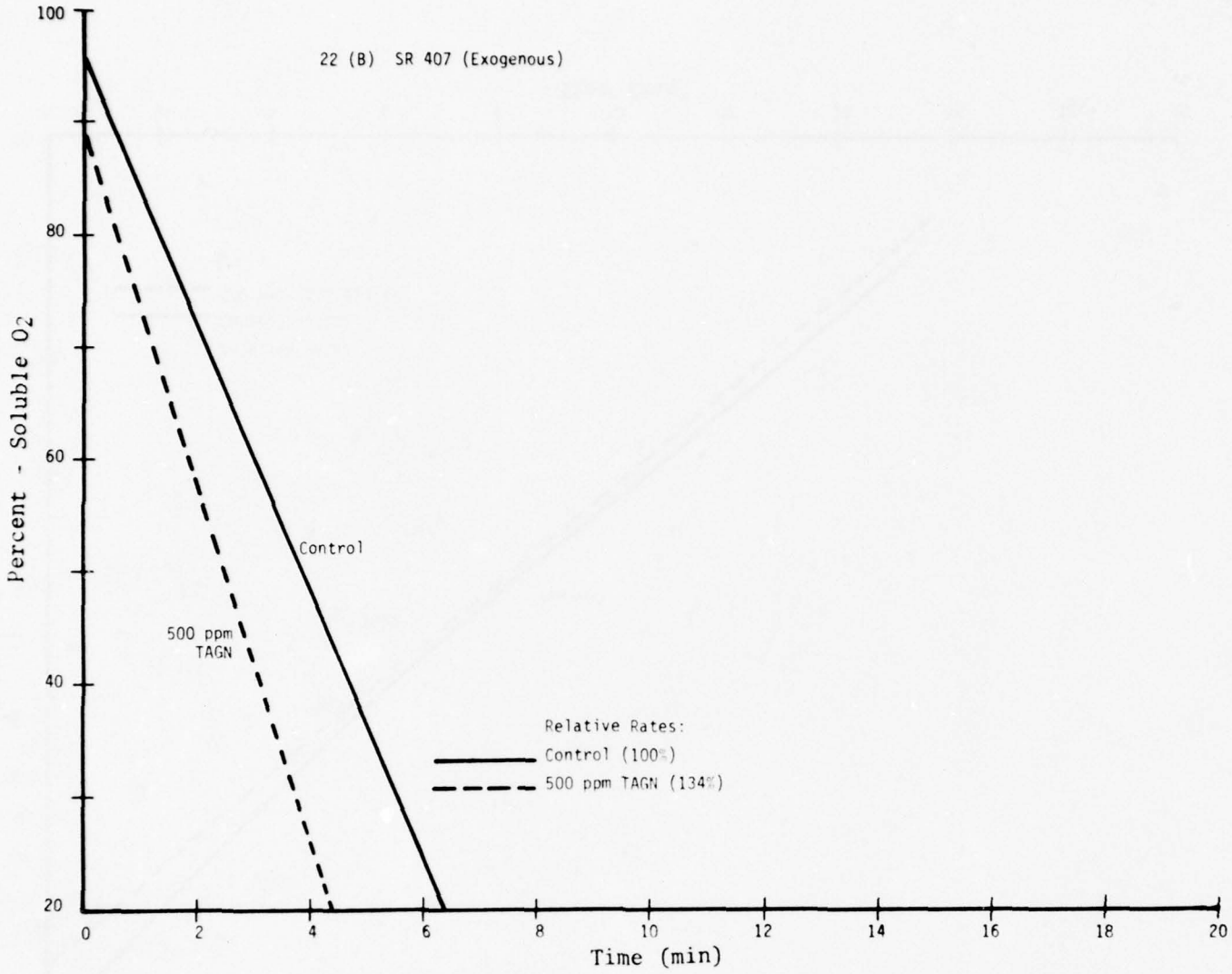
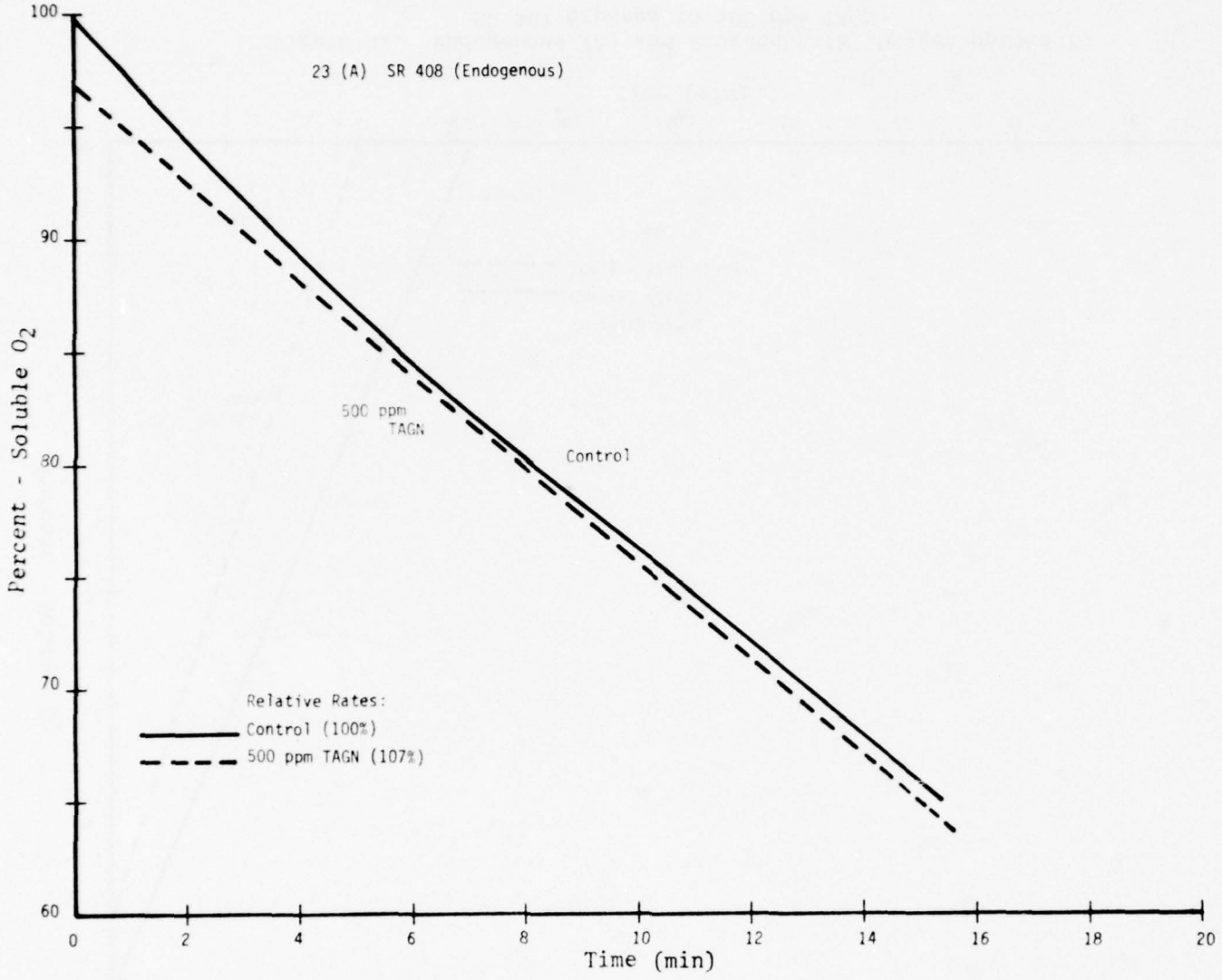


Figure 22. Endogenous (A) and Exogenous (B) Oxygen Uptake by SR 407 Exposed to 500 ppm TAGN


23 (A) SR 408 (Endogenous)
100
90
80
70
60
Percent - Soluble O_2
500 ppm TAGN
Control
Relative Rates:
Control (100%)
500 ppm TAGN (107%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

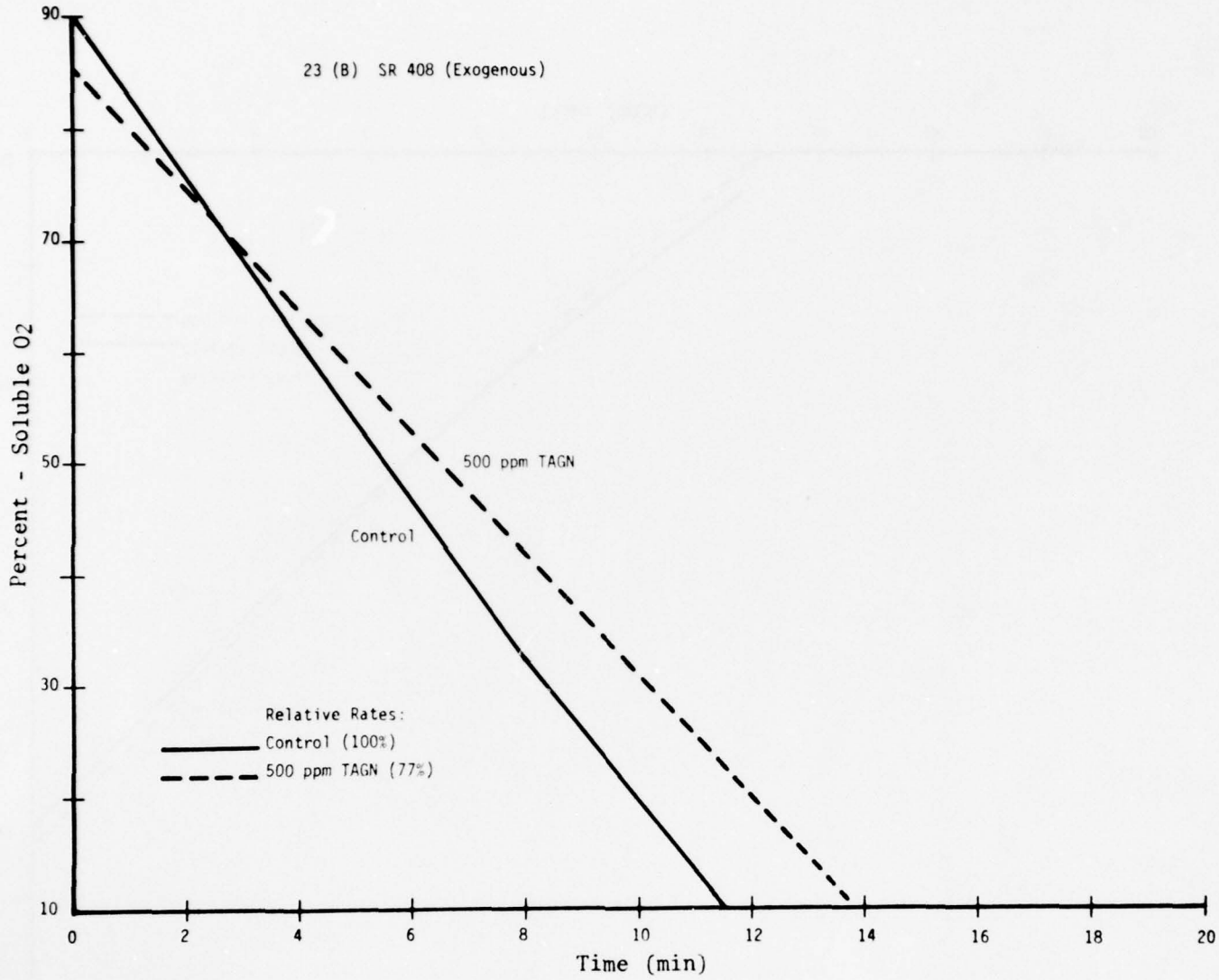
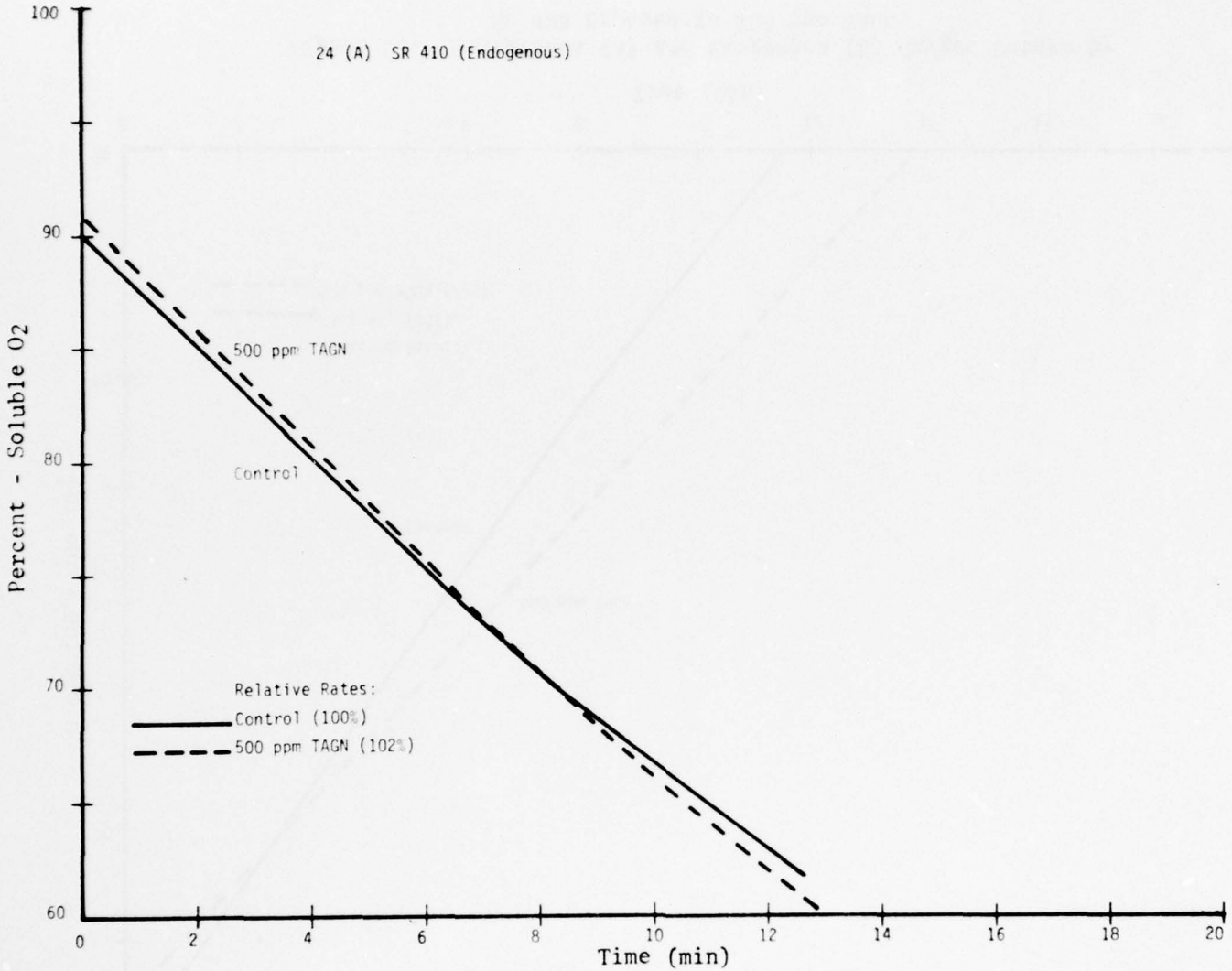


Figure 23. Endogenous (A) and Exogenous (B) Oxygen Uptake by SR 408 Exposed to 500 ppm TAGN


24 (A) SR 410 (Endogenous)
Percent - Soluble O_2
100
90
80
70
60
500 ppm TAGN
Control
Relative Rates:
Control (100%)
500 ppm TAGN (102%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

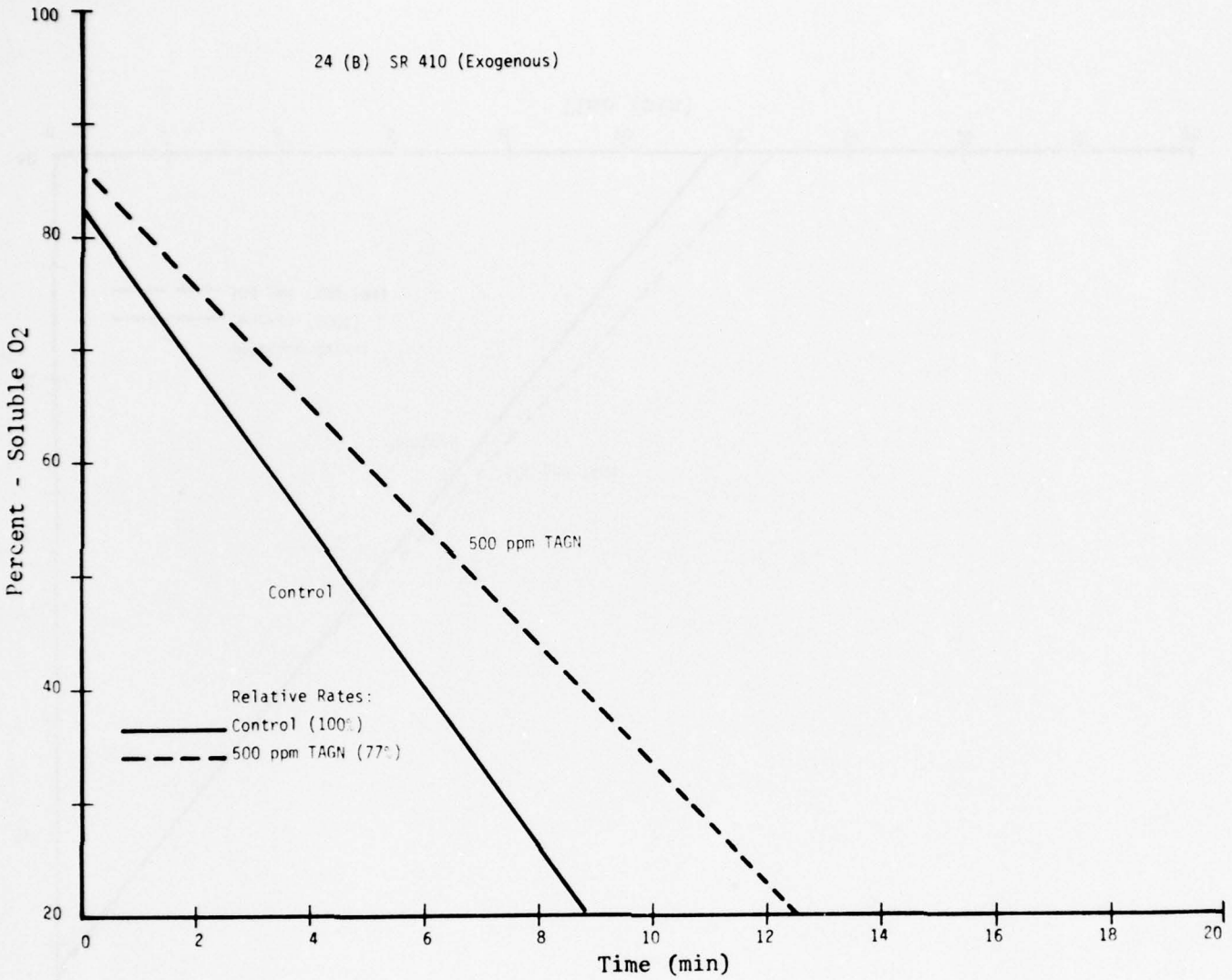
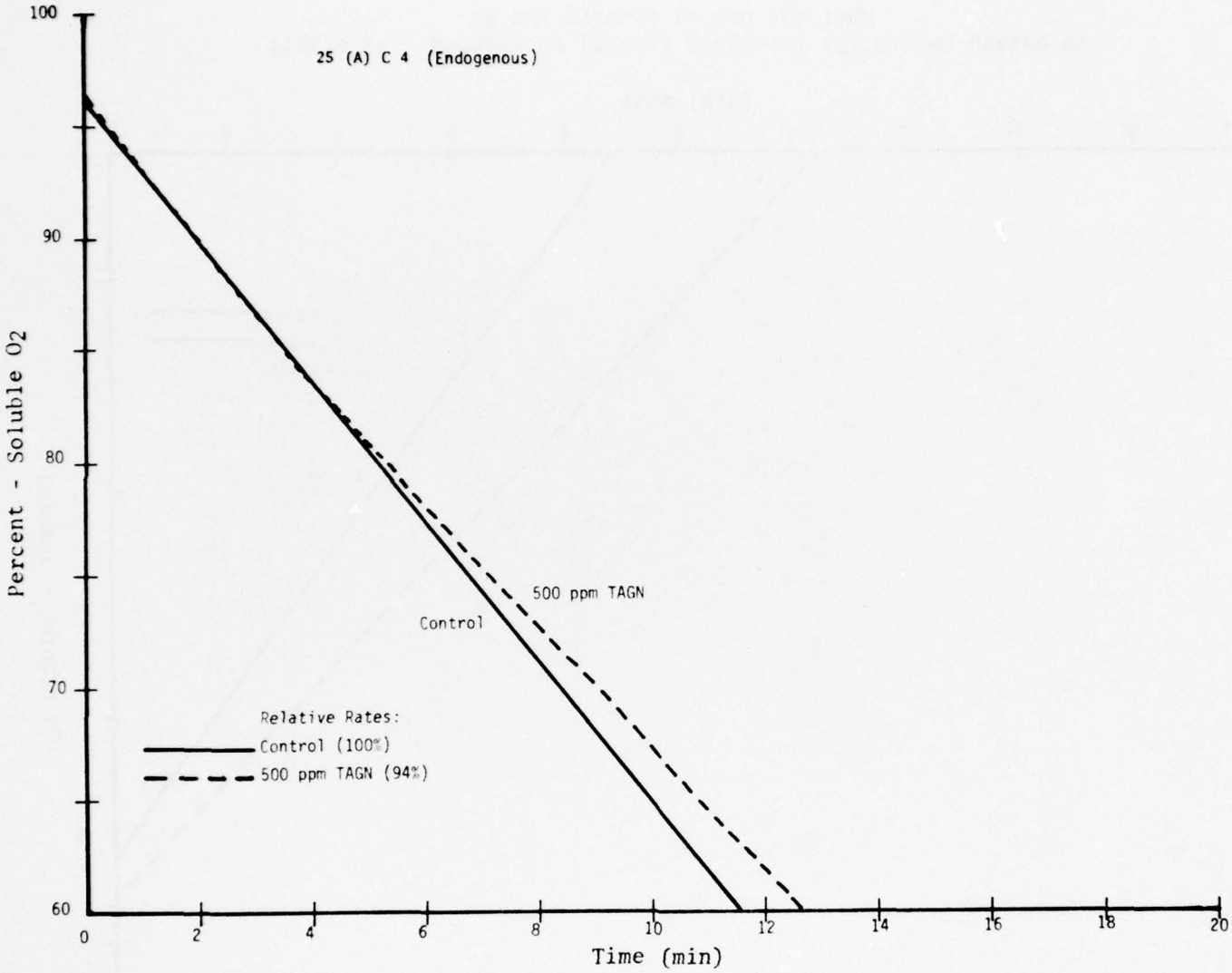


Figure 24. Endogenous (A) and Exogenous (B) Oxygen Uptake by SR 410 Exposed to 500 ppm TAGN


25 (A) C 4 (Endogenous)
100
90
80
70
60
Percent - Soluble O2
500 ppm TAGN
Control
Relative Rates:
Control (100%)
500 ppm TAGN (94%)
0
2
4
6
8
10
12
14
16
18
20
Time (min)

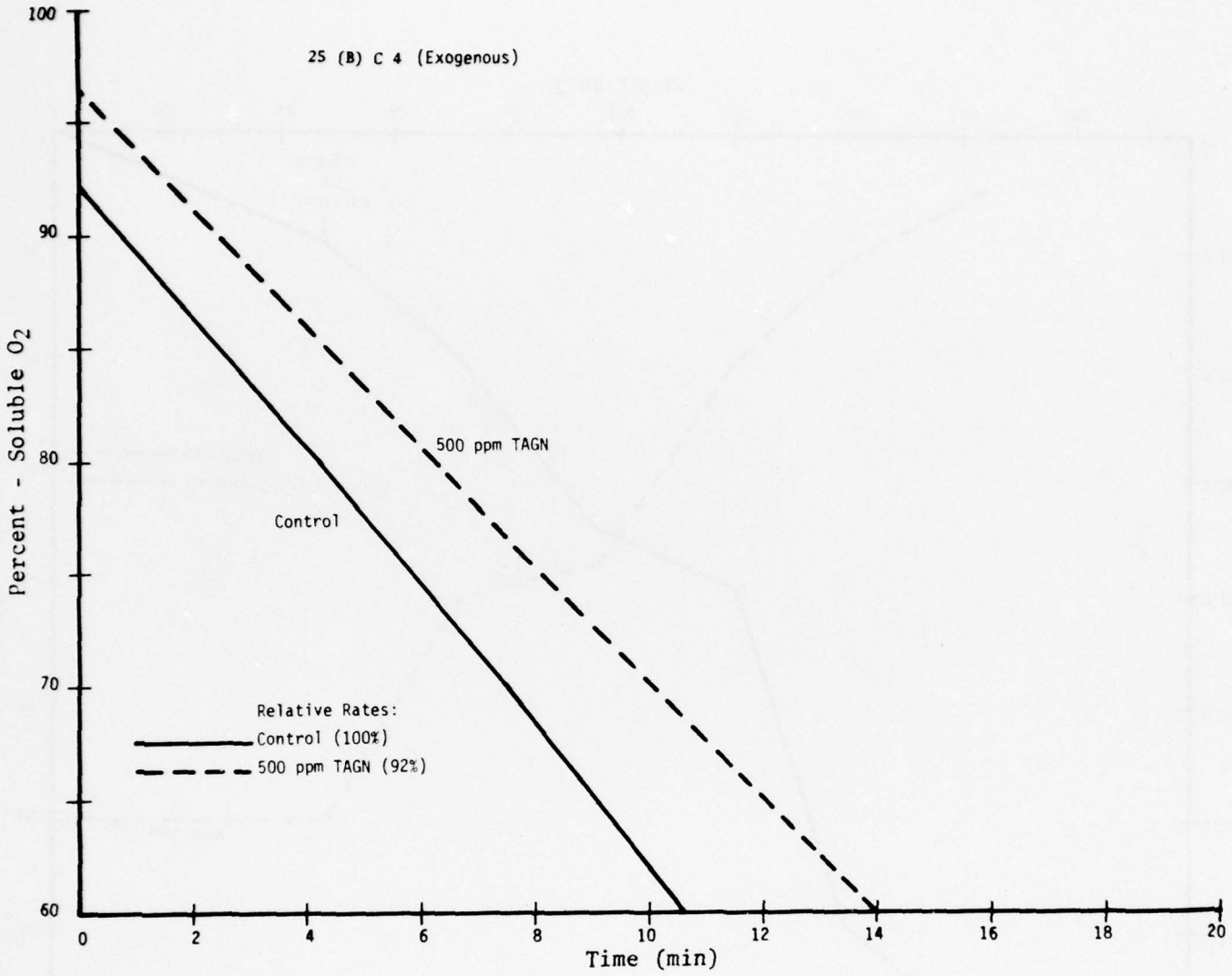
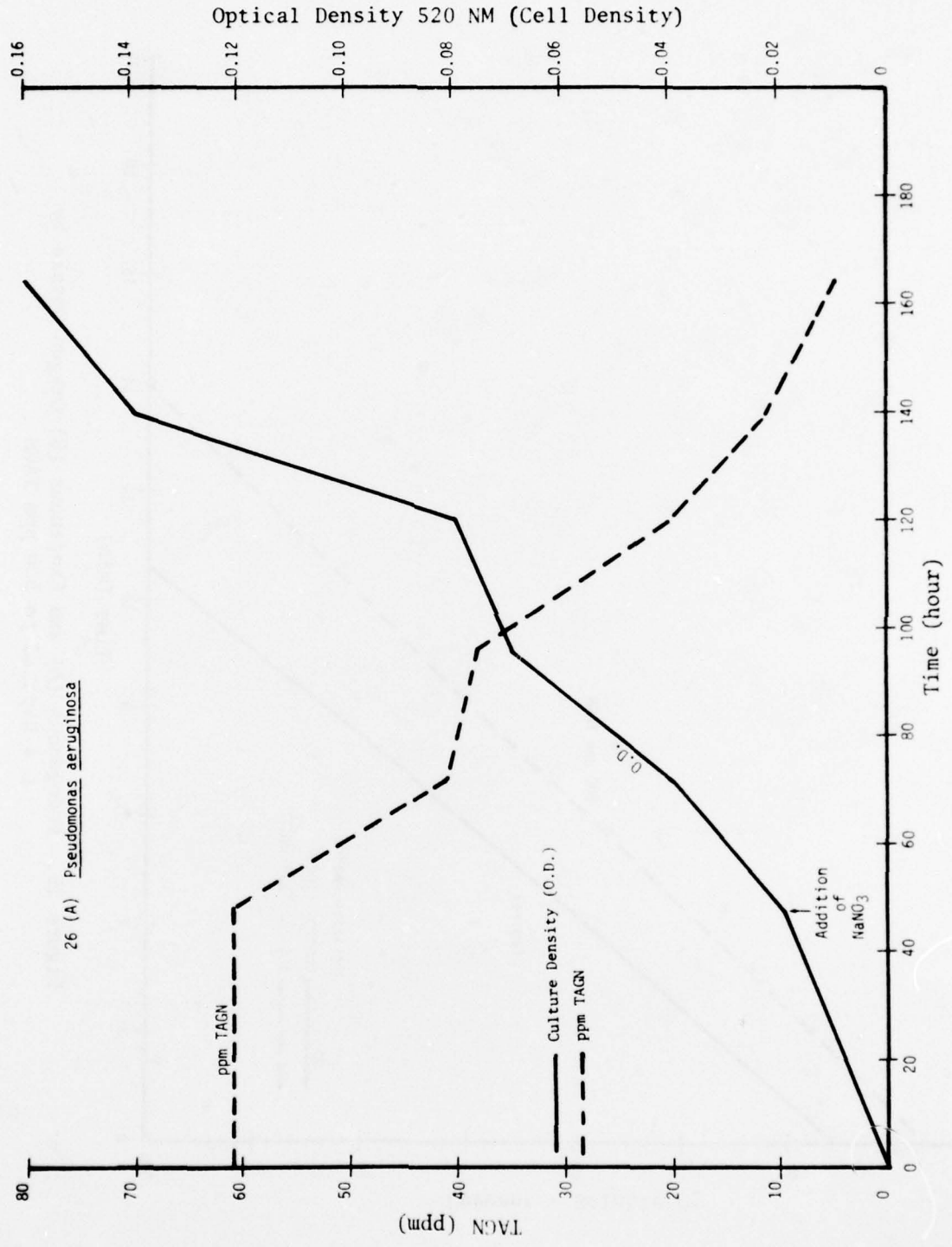


Figure 25. Endogenous (A) and Exogenous (B) Oxygen Uptake by C 4 Exposed to 500 ppm TAGN


Optical Density 520 NM (Cell Density)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
180
160
140
120
100
80
60
40
20
0
Time (hour)
26 (A) Pseudomonas aeruginosa
ppm TAGN
Culture Density (O.D.)
ppm TAGN
Addition of $NaNO_3$
0.0
80
70
60
50
40
30
20
10
0
TAGN (ppm)

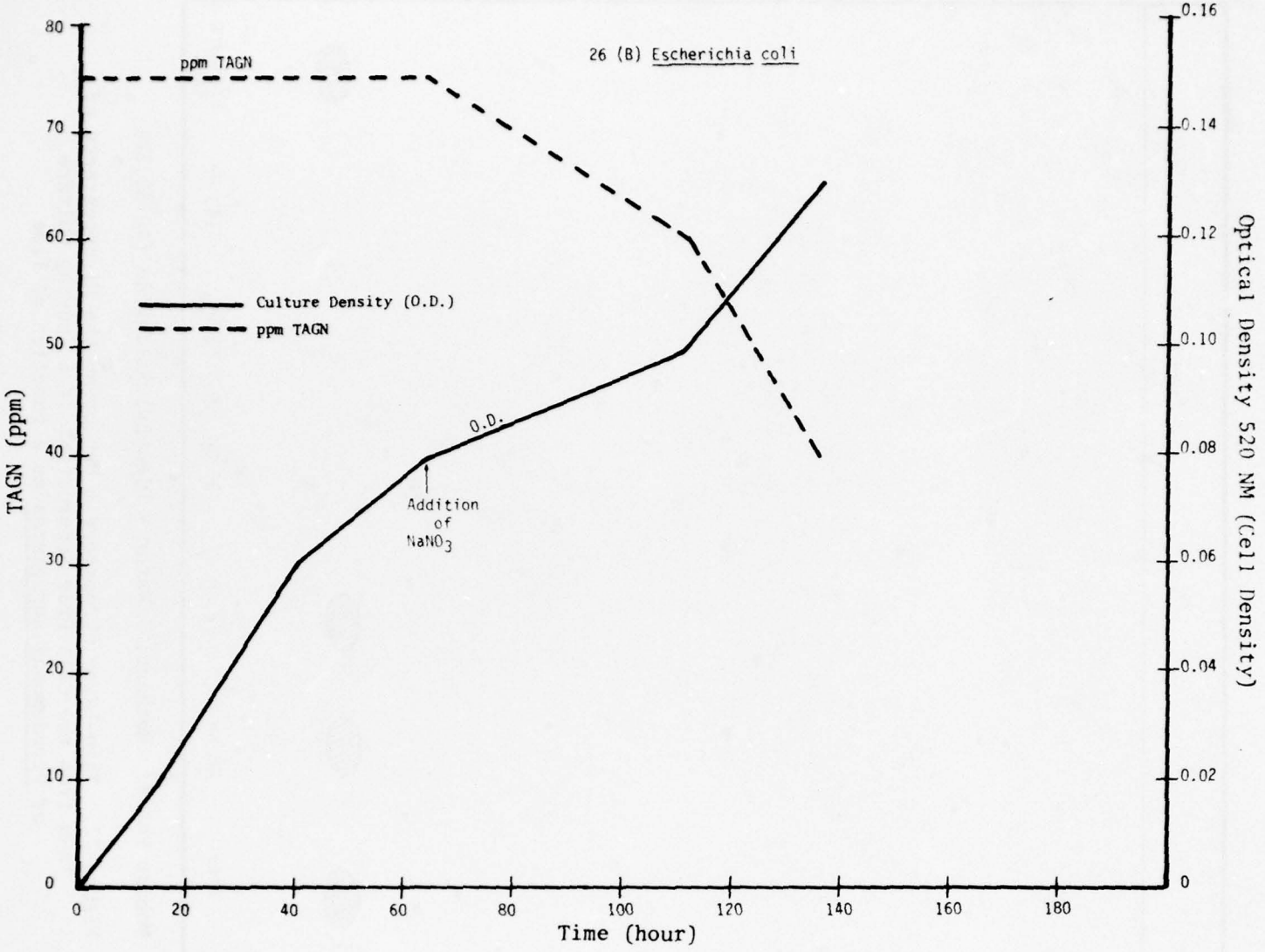
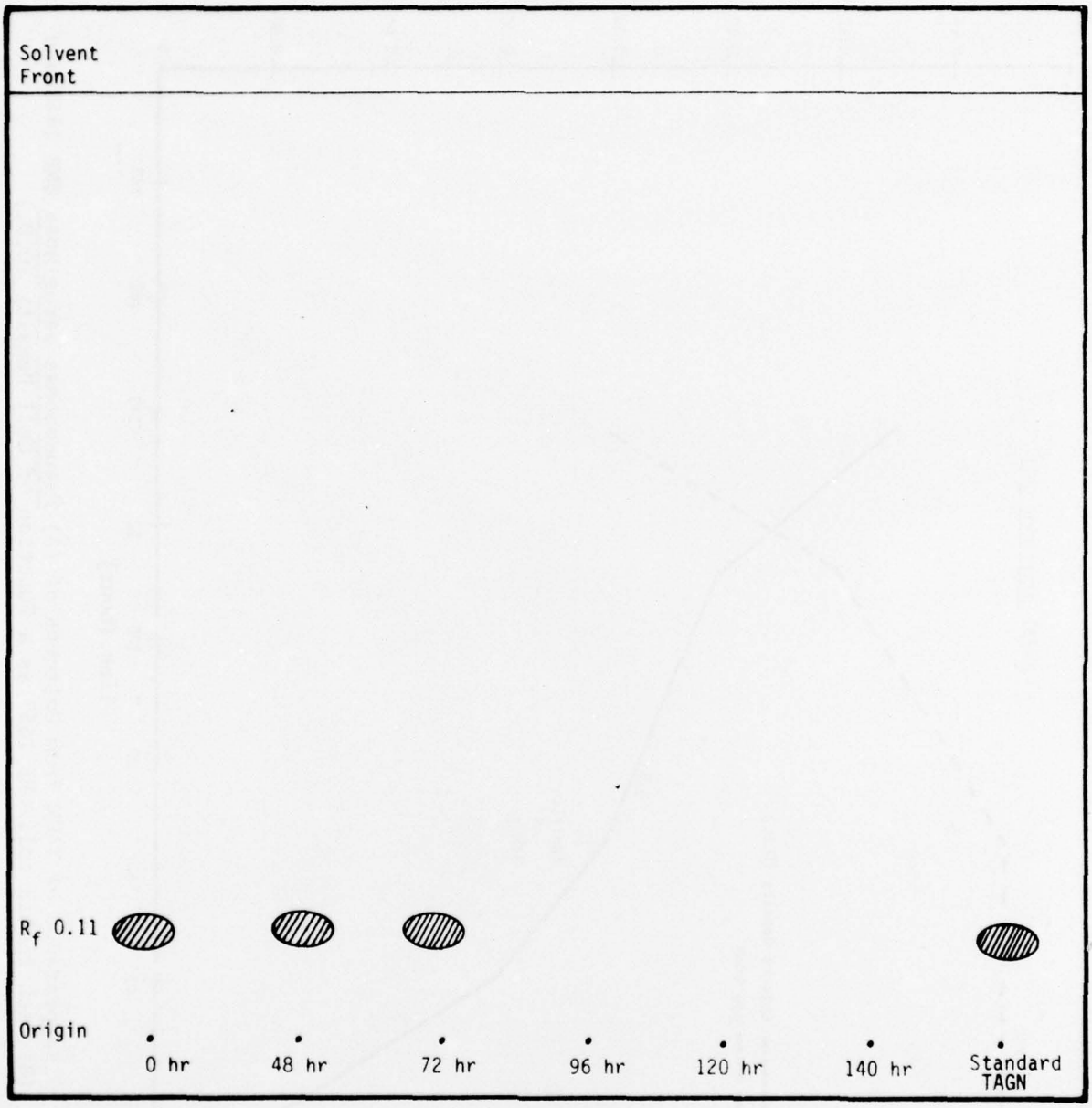


Figure 26. Disappearance of TAGN from Cultures of (A) Pseudomonas aeruginosa QMB 1468 and (B) Escherichia coli QMB 1557 as a Function of Cell Density (O.D.)



Mobile Phase: Methanol - Water - Dimethyl Sulfoxide (40:30:30)

Figure 27. Thin-Layer Chromatogram Depicting the Disappearance of TAGN from the Cell-Free Supernatant of a Growing Culture of Pseudomonas aeruginosa as a Function of Time

TABLE 1. EXPOSURE OF BACTERIAL CULTURES OBTAINED FROM US ARMY NATICK LABORATORIES TO TAGN

(N is the number of replicate samples; $\hat{\alpha}$ is the observed significance level)

Sample	Incubation (hr)	TAGN (ppm)	N	Mean	Standard Deviation	$\hat{\alpha}$
Pseudomonas aeruginosa	1	0	6	2.2×10^{7}	0.6×10^{7}	---
	1	500	5	1.8×10^{7}	0.9×10^{7}	0.114
	1	2000	6	1.4×10^{7}	0.4×10^{7}	0.011
	5	0	5	6.3×10^{7}	1.1×10^{7}	---
	5	500	6	3.8×10^{7}	1.0×10^{7}	0.004
	5	2000	4	3.2×10^{7}	0.2×10^{7}	0.002
Bacillus megaterium	1	0	6	11×10^{6}	2.0×10^{6}	---
	1	500	6	5.8×10^{6}	2.7×10^{6}	0.003
	1	2000	6	6.7×10^{6}	1.2×10^{6}	0.002
	5	0	6	2.0×10^{6}	0.7×10^{6}	---
	5	500	5	1.0×10^{6}	0.3×10^{6}	0.007
	5	2000	6	2.8×10^{6}	0.6×10^{6}	0.022
Bacillus cereus	1	0	6	2.1×10^{6}	0.6×10^{6}	---
	1	500	6	2.6×10^{6}	2.0×10^{6}	0.147
	1	2000	6	2.9×10^{6}	2.0×10^{6}	0.103
	5	0	6	2.7×10^{6}	0.7×10^{6}	---
	5	500	6	1.2×10^{6}	0.5×10^{6}	0.002
	5	2000	6	2.6×10^{6}	1.4×10^{6}	>0.2

TABLE 1. EXPOSURE OF BACTERIAL CULTURES OBTAINED FROM US ARMY NATICK LABORATORIES TO TAGN (CONCLUDED)

(N is the number of replicate samples; $\hat{\alpha}$ is the observed significance level)

Sample	Incubation (hr)	TAGN (ppm)	N	Mean	Standard Deviation	$\hat{\alpha}$
Staphylococcus aureus	1	0	6	7.3×10^7	1.5×10^7	---
	1	500	6	7.9×10^7	1.5×10^7	0.13
	1	2000	6	6.6×10^7	1.9×10^7	0.128
	5	0	6	7.7×10^7	0.5×10^7	---
	5	500	6	8.1×10^7	2.1×10^7	0.169
	5	2000	6	7.9×10^7	1.2×10^7	0.182
Serratia marcescens	1	0	6	7.0×10^7	0.4×10^7	---
	1	500	6	8.2×10^7	3.3×10^7	0.109
	1	2000	6	11×10^7	2.0×10^7	0.002
	5	0	6	8.2×10^7	2.0×10^7	---
	5	500	6	7.2×10^7	1.4×10^7	0.097
	5	2000	6	8.2×10^7	1.5×10^7	---
Escherichia coli	1	0	6	1.3×10^7	0.7×10^7	---
	1	500	6	1.6×10^7	0.9×10^7	0.138
	1	2000	6	1.5×10^7	0.8×10^7	0.002
	5	0	3	6.9×10^7	3.1×10^7	---
	5	500	6	2.8×10^7	1.5×10^7	0.042
	5	2000	6	2.1×10^7	0.7×10^7	0.031

TABLE 2. EXPOSURE OF BACTERIAL CULTURES INDIGENOUS TO EGLIN AFB, FLORIDA, TO TAGN

(N is the number of replicate samples; $\hat{\alpha}$ is the observed significance level)

Sample	Incubation (hr)	TAGN (ppm)	N	Mean	Standard Deviation	$\hat{\alpha}$
SR 409	1	0	6	1.5×10^7	0.2×10^7	---
	1	500	5	1.8×10^7	0.3×10^7	0.033
	1	2000	6	1.8×10^7	5.6×10^7	>0.2
	5	0	4	2.8×10^7	6.3×10^7	---
	5	500	6	1.8×10^7	0.1×10^7	0.194
	5	2000	6	2.3×10^7	0.8×10^7	>0.2
SR 404	1	0	5	0.8×10^6	0.4×10^6	---
	1	500	6	1.3×10^6	0.1×10^6	0.013
	1	2000	5	1.1×10^6	0.2×10^6	0.100
	5	0	4	7.0×10^6	0.6×10^6	---
	5	500	6	3.9×10^6	1.1×10^6	<0.00025
	5	2000	4	7.0×10^6	2.4×10^6	---
SR 406	1	0	6	5.6×10^7	1.0×10^7	---
	1	500	6	5.7×10^7	0.9×10^7	>0.20
	1	2000	6	8.0×10^7	0.6×10^7	0.001
	5	0	5	8.7×10^7	1.0×10^7	---
	5	500	5	8.4×10^7	1.8×10^7	0.191
	5	2000	6	7.6×10^7	1.9×10^7	0.076

TABLE 2. EXPOSURE OF BACTERIAL CULTURES INDIGENOUS TO EGLIN AFB, FLORIDA, TO TAGN (CONTINUED)

(N is the number of replicate samples; $\hat{\alpha}$ is the observed significance level)

Sample	Incubation (hr)	TAGN (ppm)	N	Mean	Standard Deviation	$\hat{\alpha}$
SR 402	1	0	6	4.3×10^7	1.0×10^7	---
	1	500	6	4.8×10^7	2.0×10^7	0.154
	1	2000	6	5.8×10^7	1.5×10^7	0.024
	5	0	6	5.9×10^7	0.9×10^7	---
	5	500	6	10×10^7	1.0×10^7	<0.00025
	5	2000	6	3.5×10^7	0.9×10^7	0.002
SR 407	1	0	6	4.8×10^7	1.9×10^7	---
	1	500	6	4.1×10^7	0.8×10^7	0.114
	1	2000	6	7.4×10^7	1.1×10^7	0.009
	5	0	6	3.7×10^7	1.8×10^7	---
	5	500	6	5.2×10^7	2.1×10^7	0.061
	5	2000	6	2.9×10^7	0.5×10^7	0.092
SR 408	1	0	6	2.4×10^7	0.6×10^7	---
	1	500	6	1.9×10^7	0.2×10^7	0.028
	1	2000	6	3.1×10^7	0.6×10^7	0.029
	5	0	6	3.2×10^7	0.7×10^7	---
	5	500	6	2.8×10^7	0.4×10^7	0.083
	5	2000	6	3.0×10^7	0.5×10^7	0.150

TABLE 2. EXPOSURE OF BACTERIAL CULTURES INDIGENOUS TO EGLIN AFB, FLORIDA, TO TAGN (CONCLUDED)

(N is the number of replicate samples; $\hat{\alpha}$ is the observed significance level)

Sample	Incubation (hr)	TAGN (ppm)	N	Mean	Standard Deviation	$\hat{\alpha}$
SR 405	1	0	5	1.0×10^7	0.1×10^7	---
	1	500	6	1.2×10^7	0.4×10^7	0.080
	1	2000	6	1.0×10^7	0.2×10^7	---
	5	0	6	1.6×10^7	0.3×10^7	---
	5	500	6	1.0×10^7	0.4×10^7	0.009
	5	2000	6	0.3×10^7	0.2×10^7	<0.00025

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